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(54) Title: REGULATION OF HUMAN SPHINGOSINE KINASE-LIKE PROTEIN

(57) Abstract: Reagents which regulate human sphingosine kinase-like protein activity and reagents which bind to human sphingosine kinase-like protein gene products can be used to regulate intracellular signaling intracellular signaling and consequently cell proliferation and apoptosis. Such regulation is particularly useful for treating cancer, allergies including but not limited to asthma, autoimmune diseases such as rheumatoid arthritis, and central and peripheral nervous system disorders, such as Parkinson's disease.

REGULATION OF HUMAN SPHINGOSINE KINASE-LIKE PROTEIN

This application claims the benefit of and incorporates by reference co-pending provisional application Serial No. 60/238,005 filed October 6, 2000.

TECHNICAL FIELD OF THE INVENTION

The invention relates to the area of regulation of intracellular signaling. More particularly, the invention relates to the regulation of human sphingosine kinase-like protein activity to increase or decrease intracellular signaling.

BACKGROUND OF THE INVENTION

Sphingosine kinase is found in a wide variety of human tissues, including the lung, liver, spleen, kidney, brain, testis, and hematopoietic system. Sphingosine kinase forms sphingosine-1-phosphate (SPP) from sphingosine. The sphingolipid metabolites, sphingosine, SPP and sphingosylphosphorylcholine (SPC) are emerging as a new class of intracellular second messengers with a wide spectrum of activity in cell growth regulation and signal transduction. It is known that ceramide is an important regulatory participant in programmed cell death (apoptosis) induced by tumor-necrosis factor-β (TNFβ) and Fas ligand, members of the TNF superfamily. Conversely, sphingosine and sphingosine-1-phosphate (SPP), which are metabolites of ceramide, induce mitogenesis and have been implicated as second messengers in cellular proliferation induced by platelet-derived growth factor and serum. See U.S. Patent 5,712,262.

SPP prevents the appearance of the key features of apoptosis, namely, intra-nucleosomal DNA fragmentation and morphological changes, which result from increased concentrations of ceramide. Furthermore, inhibition of ceramide-mediated apoptosis by activation of protein kinase C results from stimulation of sphingosine

kinase and the concomitant increase in intracellular sphingosine-1-phosphate. Finally, SPP not only stimulates the extracellular signal-regulated kinase (ERK) pathway, but also counteracts the ceramide-induced activation of stress-activated protein kinase. Thus, the balance between the intracellular levels of ceramide and SPP and their regulatory effects on different family members of mitogen-activated protein kinases determines the fate of the cell.

Inappropriate sphingosine kinase activity has been implicated in several diseases. For example, elevated levels of SPP are correlated with resistance to apoptosis seen in autoimmune diseases such as rheumatoid arthritis. Decreased levels of SPP are correlated with inhibition of cellular proliferation

Thus, there is a need in the art for identifying new sphingosine kinase-like proteins and methods of regulating intracellular signaling and apoptosis.

SUMMARY OF THE INVENTION

It is an object of the invention to provide reagents and methods of regulating intracellular signaling. These and other objects of the invention are provided by one or more of the embodiments described below.

One embodiment of the invention is a sphingosine kinase-like protein polypeptide comprising an amino acid sequence selected from the group consisting of:

amino acid sequences which are at least about 50% identical to the amino acid sequence shown in SEQ ID NO: 2;

the amino acid sequence shown in SEQ ID NO: 2;

amino acid sequences which are at least about 50% identical to the amino acid sequence shown in SEQ ID NO: 10;

the amino acid sequence shown in SEQ ID NO: 10;

amino acid sequences which are at least about 50% identical to the amino acid sequence shown in SEQ ID NO: 11; and

the amino acid sequence shown in SEQ ID NO: 11.

Yet another embodiment of the invention is a method of screening for agents which decrease extracellular matrix degradation. A test compound is contacted with a sphingosine kinase-like protein polypeptide comprising an amino acid sequence selected from the group consisting of:

amino acid sequences which are at least about 50% identical to the amino acid sequence shown in SEQ ID NO: 2;

the amino acid sequence shown in SEQ ID NO: 2;

amino acid sequences which are at least about 50% identical to the amino acid sequence shown in SEQ ID NO: 10;

the amino acid sequence shown in SEQ ID NO: 10;

amino acid sequences which are at least about 50% identical to the amino acid sequence shown in SEQ ID NO: 11; and

the amino acid sequence shown in SEQ ID NO: 11.

Binding between the test compound and the sphingosine kinase-like protein polypeptide is detected. A test compound which binds to the sphingosine kinase-like protein polypeptide is thereby identified as a potential agent for decreasing

extracellular matrix degradation. The agent can work by decreasing the activity of the sphingosine kinase-like protein.

Another embodiment of the invention is a method of screening for agents which decrease extracellular matrix degradation. A test compound is contacted with a polynucleotide encoding a sphingosine kinase-like protein polypeptide, wherein the polynucleotide comprises a nucleotide sequence selected from the group consisting of:

nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO: 1;

the nucleotide sequence shown in SEQ ID NO: 1;

nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO: 9; and

the nucleotide sequence shown in SEQ ID NO: 9.

Binding of the test compound to the polynucleotide is detected. A test compound which binds to the polynucleotide is identified as a potential agent for decreasing extracellular matrix degradation. The agent can work by decreasing the amount of the sphingosine kinase-like protein through interacting with the sphingosine kinase-like protein mRNA.

Another embodiment of the invention is a method of screening for agents which regulate extracellular matrix degradation. A test compound is contacted with a sphingosine kinase-like protein polypeptide comprising an amino acid sequence selected from the group consisting of:

amino acid sequences which are at least about 50% identical to the amino acid sequence shown in SEQ ID NO: 2;

the amino acid sequence shown in SEQ ID NO: 2;

amino acid sequences which are at least about 50% identical to the amino acid sequence shown in SEQ ID NO: 10;

the amino acid sequence shown in SEQ ID NO: 10;

amino acid sequences which are at least about 50% identical to the amino acid sequence shown in SEQ ID NO: 11; and

the amino acid sequence shown in SEQ ID NO: 11.

A sphingosine kinase-like protein activity of the polypeptide is detected. A test compound which increases sphingosine kinase-like protein activity of the polypeptide relative to sphingosine kinase-like protein activity in the absence of the test compound is thereby identified as a potential agent for increasing extracellular matrix degradation. A test compound which decreases sphingosine kinase-like protein activity of the polypeptide relative to sphingosine kinase-like protein activity in the absence of the test compound is thereby identified as a potential agent for decreasing extracellular matrix degradation.

Even another embodiment of the invention is a method of screening for agents which decrease extracellular matrix degradation. A test compound is contacted with a sphingosine kinase-like protein product of a polynucleotide which comprises a nucleotide sequence selected from the group consisting of:

nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO: 1;

the nucleotide sequence shown in SEQ ID NO: 1;

nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO: 9; and

the nucleotide sequence shown in SEQ ID NO: 9.

Binding of the test compound to the sphingosine kinase-like protein product is detected. A test compound which binds to the sphingosine kinase-like protein product is thereby identified as a potential agent for decreasing extracellular matrix degradation.

Still another embodiment of the invention is a method of reducing extracellular matrix degradation. A cell is contacted with a reagent which specifically binds to a polynucleotide encoding a sphingosine kinase-like protein polypeptide or the product encoded by the polynucleotide, wherein the polynucleotide comprises a nucleotide sequence selected from the group consisting of:

nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO: 1;

the nucleotide sequence shown in SEQ ID NO: 1;

nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO: 9; and

the nucleotide sequence shown in SEQ ID NO: 9.

Sphingosine kinase-like protein activity in the cell is thereby decreased.

The invention thus provides reagents and methods for regulating intracellular signaling which can be used, *inter alia*, to suppress metastatic activity and proliferation of malignant cells and to treat autoimmune diseases, allergies including but not limited to asthma, and central and peripheral nervous system disorders.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the DNA-sequence encoding a sphingosine kinase-like protein Polypeptide (SEQ ID NO: 1).

Fig. 2 shows the amino acid sequence deduced from the DNA-sequence of Fig.1 (SEQ ID NO: 2).

Fig. 3 shows the amino acid sequence of the protein identified by EMBL Accession No. AF245447 (SEQ ID NO: 3).

Fig. 4 shows the DNA-sequence encoding a sphingosine kinase-like protein Polypeptide (SEQ ID NO: 4).

Fig. 5 shows the DNA-sequence encoding a sphingosine kinase-like protein Polypeptide (SEQ ID NO: 5).

Fig. 6 shows the DNA-sequence encoding a sphingosine kinase-like protein Polypeptide (SEQ ID NO: 6).

Fig. 7 shows the DNA-sequence encoding a sphingosine kinase-like protein Polypeptide (SEQ ID NO: 7).

Fig. 8 shows the DNA-sequence encoding a sphingosine kinase-like protein Polypeptide (SEQ ID NO: 8).

Fig. 9 shows the DNA-sequence encoding a sphingosine kinase-like protein Polypeptide (SEQ ID NO: 9).

Fig. 10 shows the amino acid sequence deduced from the DNA-sequence of Fig. 9 (SEQ ID NO: 10).

Fig. 11 shows the amino acid sequence deduced from the DNA-sequence of Fig. 9 (SEQ ID NO: 11)

Fig. 12 shows the BLASTP alignment of human sphingosine kinase-like protein (SEQ ID NO: 2) against SEQ ID NO: 3.

Fig. 13 shows the amino acid sequence of human sphingosine kinase-like protein polypeptide.

Fig. 14 shows the expression profiling of sphingosine kinase-like protein polypeptide (SEQ ID NO: 10) whole body screen.

Fig. 15 shows the expression profiling of sphingosine kinase-like protein polypeptide (SEQ ID NO: 10), blood/lung screen.

DETAILED DESCRIPTION OF THE INVENTION

The invention relates to an isolated polynucleotide encoding a sphingosine kinase-like protein polypeptide and being selected from the group consisting of:

a) a polynucleotide encoding a sphingosine kinase-like protein polypeptide comprising an amino acid sequence selected from the group consisting of:

amino acid sequences which are at least about 50% identical to the amino acid sequence shown in SEQ ID NO: 2;

the amino acid sequence shown in SEQ ID NO: 2;
amino acid sequences which are at least about 50% identical to
the amino acid sequence shown in SEQ ID NO: 10;
the amino acid sequence shown in SEQ ID NO: 10;
amino acid sequences which are at least about 50% identical to
the amino acid sequence shown in SEQ ID NO: 11; and
the amino acid sequence shown in SEQ ID NO: 11;

b) a polynucleotide comprising the sequence of SEQ ID NOS: 1 or 9;

c) a polynucleotide which hybridizes under stringent conditions to a polynucleotide specified in (a) and (b);

d) a polynucleotide the sequence of which deviates from the polynucleotide sequences specified in (a) to (c) due to the degeneration of the genetic code; and

e) a polynucleotide which represents a fragment, derivative or allelic variation of a polynucleotide sequence specified in (a) to (d).

Furthermore, it has been discovered by the present applicant that a novel protein, particularly a human sphingosine kinase-like protein is a discovery of the present invention. Human sphingosine kinase-like protein as shown in SEQ ID NO: 2 is 28% identical over 232 amino acids to the *Homo sapiens* protein identified by EMBL Accession No. AF245447 (SEQ ID NO: 3) and annotated as a putative amine oxidase (Fig. 12). Human sphingosine kinase-like protein contains a diacylglycerol kinase domain which is shown in bold in Fig. 13.

A coding sequence for SEQ ID NO: 2 is shown in SEQ ID NO: 1. A coding sequence for SEQ ID NOS: 10 and 11 is shown in SEQ ID NO: 9. Related ESTs

(SEQ ID NOS: 4-8) are expressed in germinal center B lymphocytes, T-lymphocytes, embryonic tissue, neuroblastomas, liver, ovary, brain, and kidney.

Human sphingosine kinase-like protein is expected to be useful for the same purposes as previously identified sphingosine kinase (*see* Liu, *et al.*, *J. Biol. Chem. 275*, 19513-20, 2000). Regulators of a human sphingosine kinase-like protein can be used to regulate intracellular signaling. Human sphingosine kinase-like protein is expected to be especially useful for treating cancer, allergies including but not limited to asthma, central and peripheral nervous system disorders, and autoimmune disease.

Polypeptides

Sphingosine kinase-like protein polypeptides according to the invention comprise an amino acid sequence as shown in SEQ ID NO: 2, a portion of SEQ ID NO: 2 comprising at least 6, 10, 15, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 320, or 326 contiguous amino acids, or a biologically active variant of the amino acid sequence shown in SEQ ID NO: 2, as defined below. Sphingosine kinase-like protein polypeptides according to the invention comprise an amino acid sequence as shown in SEQ ID NO: 10, a portion of SEQ ID NO: 10 comprising at least 6, 10, 15, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 525, or 537 contiguous amino acids, or a biologically active variant of the amino acid sequence shown in SEQ ID NO: 10, as defined below. Sphingosine kinase-like protein polypeptides according to the invention comprise an amino acid sequence as shown in SEQ ID NO: 11, a portion of SEQ ID NO: 11 comprising at least 6, 10, 15, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 525, 550, or 562 contiguous amino acids, or a biologically active variant of the amino acid sequence shown in SEQ ID NO: 11, as defined below. A sphingosine kinase-like protein polypeptide of the invention therefore can be a portion of a sphingosine kinase-like protein molecule, a full-length

sphingosine kinase-like protein molecule, or a fusion protein comprising all or a portion of a sphingosine kinase-like protein molecule.

Biologically Active Variants

Sphingosine kinase-like protein variants which are biologically active, *i.e.*, retain a sphingosine kinase-like protein activity, also are sphingosine kinase-like protein polypeptides. Preferably, naturally or non-naturally occurring sphingosine kinase-like protein variants have amino acid sequences which are at least about 30, 35, 40, 45, 50, 55, 60, 65, 70, preferably about 75, 90, 96, or 98% identical to an amino acid sequence shown in SEQ ID NO: 2. Percent identity between a putative sphingosine kinase-like protein variant and an amino acid sequence of SEQ ID NO: 2 is determined using the Blast2 alignment program.

Variations in percent identity can be due, for example, to amino acid substitutions, insertions, or deletions. Amino acid substitutions are defined as one for one amino acid replacements. They are conservative in nature when the substituted amino acid has similar structural and/or chemical properties. Examples of conservative replacements are substitution of a leucine with an isoleucine or valine, an aspartate with a glutamate, or a threonine with a serine.

Amino acid insertions or deletions are changes to or within an amino acid sequence. They typically fall in the range of about 1 to 5 amino acids. Guidance in determining which amino acid residues can be substituted, inserted, or deleted without abolishing biological or immunological activity can be found using computer programs well known in the art, such as DNASTAR software. Whether an amino acid change results in a biologically active sphingosine kinase-like protein polypeptide can readily be determined by assaying for sphingosine kinase activity, as is known in the art and described, for example, in Liu, *et al.*, *J. Biol. Chem. 275*, 19513-19520, 2000.

Fusion Proteins

Fusion proteins are useful for generating antibodies against sphingosine kinase-like protein amino acid sequences and for use in various assay systems. For example, fusion proteins can be used to identify proteins which interact with portions of a sphingosine kinase-like protein polypeptide, including its active site and fibronectin domains. Methods such as protein affinity chromatography or library-based assays for protein-protein interactions, such as the yeast two-hybrid or phage display systems, can be used for this purpose. Such methods are well known in the art and also can be used as drug screens.

A sphingosine kinase-like protein fusion protein comprises two protein segments fused together by means of a peptide bond. Contiguous amino acids for use in a fusion protein can be selected from the amino acid sequence shown in SEQ ID NO: 2 or from a biologically active variants of those sequences, such as those described above. For example, the first protein segment can comprise at least 6, 10, 15, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, or 340 or more contiguous amino acids of SEQ ID NO: 2 or a biologically active variant. Sphingosine kinase-like protein polypeptides according to the invention comprise an amino acid sequence as shown in SEQ ID NO: 10, a portion of SEQ ID NO: 10 comprising at least 6, 10, 15, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 525, or 537 contiguous amino acids, or a biologically active variant of the amino acid sequence shown in SEQ ID NO: 10, as defined above. Sphingosine kinase-like protein polypeptides according to the invention comprise an amino acid sequence as shown in SEQ ID NO: 11, a portion of SEQ ID NO: 11 comprising at least 6, 10, 15, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 525, 550, or 562 contiguous amino acids, or a biologically active variant of the amino acid sequence shown in SEQ ID NO: 11, as defined above. Preferably, a fusion protein comprises the active site of the protease and/or one or both of the fibronectin domains. The first protein segment also can comprise full-length sphingosine kinase-like protein.

The second protein segment can be a full-length protein or a protein fragment or polypeptide. Proteins commonly used in fusion protein construction include β-galactosidase, β-glucuronidase, green fluorescent protein (GFP), autofluorescent proteins, including blue fluorescent protein (BFP), glutathione-S-transferase (GST), luciferase, horseradish peroxidase (HRP), and chloramphenicol acetyltransferase (CAT). Additionally, epitope tags are used in fusion protein constructions, including histidine (His) tags, FLAG tags, influenza hemagglutinin (HA) tags, Myc tags, VSV-G tags, and thioredoxin (Trx) tags. Other fusion constructions can include maltose binding protein (MBP), S-tag, Lex a DNA binding domain (DBD) fusions, GAL4 DNA binding domain fusions, and herpes simplex virus (HSV) BP16 protein fusions. A fusion protein also can be engineered to contain a cleavage site located between the sphingosine kinase-like protein polypeptide-encoding sequence and the heterologous protein sequence, so that the sphingosine kinase-like protein polypeptide can be cleaved and purified away from the heterologous moiety.

A fusion protein can be synthesized chemically, as is known in the art. Preferably, a fusion protein is produced by covalently linking two protein segments or by standard procedures in the art of molecular biology. Recombinant DNA methods can be used to prepare fusion proteins, for example, by making a DNA construct which comprises sphingosine kinase-like protein coding sequences disclosed herein in proper reading frame with nucleotides encoding the second protein segment and expressing the DNA construct in a host cell, as is known in the art. Many kits for constructing fusion proteins are available from companies such as Promega Corporation (Madison, WI), Stratagene (La Jolla, CA), CLONTECH (Mountain View, CA), Santa Cruz Biotechnology (Santa Cruz, CA), MBL International Corporation (MIC; Watertown, MA), and Quantum Biotechnologies (Montreal, Canada; 1-888-DNA-KITS).

Identification of Species Homologs

Species homologs of human sphingosine kinase-like protein can be obtained using sphingosine kinase-like protein polynucleotides (described below) to make suitable probes or primers to screening cDNA expression libraries from other species, such as mice, monkeys, or yeast, identifying cDNAs which encode homologs of sphingosine kinase-like protein, and expressing the cDNAs as is known in the art.

Polynucleotides

A sphingosine kinase-like protein polynucleotide can be single- or double-stranded and comprises a coding sequence or the complement of a coding sequence for a sphingosine kinase-like protein polypeptide. A partial coding sequence of a sphingosine kinase-like protein polynucleotide is shown in SEQ ID NOS: 1 and 9.

Degenerate nucleotide sequences encoding human sphingosine kinase-like protein polypeptides, as well as homologous nucleotide sequences which are at least about 50, 55, 60, 65, 70, preferably about 75, 90, 96, or 98% identical to the sphingosine kinase-like protein coding sequences nucleotide sequence shown in SEQ ID NO: 1 also are sphingosine kinase-like protein polynucleotides. Percent sequence identity between the sequences of two polynucleotides is determined using computer programs such as ALIGN which employ the FASTA algorithm, using an affine gap search with a gap open penalty of -12 and a gap extension penalty of -2. Complementary DNA (cDNA) molecules, species homologs, and variants of sphingosine kinase-like protein polynucleotides which encode biologically active sphingosine kinase-like protein polypeptides also are sphingosine kinase-like protein polynucleotides.

Identification of Variants and Homologs

Variants and homologs of the sphingosine kinase-like protein polynucleotides disclosed above also are sphingosine kinase-like protein polynucleotides. Typically, homologous sphingosine kinase-like protein polynucleotide sequences can be identified by hybridization of candidate polynucleotides to known sphingosine kinase-like protein polynucleotides under stringent conditions, as is known in the art. For example, using the following wash conditions--2X SSC (0.3 M NaCl, 0.03 M sodium citrate, pH 7.0), 0.1% SDS, room temperature twice, 30 minutes each; then 2X SSC, 0.1% SDS, 50°C once, 30 minutes; then 2X SSC, room temperature twice, 10 minutes each--homologous sequences can be identified which contain at most about 25-30% basepair mismatches. More preferably, homologous nucleic acid strands contain 15-25% basepair mismatches, even more preferably 5-15% basepair mismatches.

Species homologs of the sphingosine kinase-like protein polynucleotides disclosed herein can be identified by making suitable probes or primers and screening cDNA expression libraries from other species, such as mice, monkeys, or yeast. Human variants of sphingosine kinase-like protein polynucleotides can be identified, for example, by screening human cDNA expression libraries. It is well known that the T_m of a double- stranded DNA decreases by 1-1.5°C with every 1% decrease in homology (Bonner *et al.*, *J. Mol. Biol. 81*, 123 (1973). Variants of human sphingosine kinase-like protein polynucleotides or sphingosine kinase-like protein polynucleotides of other species can therefore be identified, for example, by hybridizing a putative homologous sphingosine kinase-like protein polynucleotide with a polynucleotide having a nucleotide sequence of SEQ ID NOS: 1 and 9. The melting temperature of the test hybrid is compared with the melting temperature of a hybrid comprising sphingosine kinase-like protein polynucleotides having perfectly complementary nucleotide sequences, and the number or percent of basepair mismatches within the test hybrid is calculated.

Nucleotide sequences which hybridize to sphingosine kinase-like protein polynucleotides or their complements following stringent hybridization and/or wash conditions are also sphingosine kinase-like protein polynucleotides. Stringent wash conditions are well known and understood in the art and are disclosed, for example, in Sambrook *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL, 2d ed., 1989, at pages 9.50-9.51.

Typically, for stringent hybridization conditions a combination of temperature and salt concentration should be chosen that is approximately 12-20°C below the calculated T_m of the hybrid under study. The T_m of a hybrid between a sphingosine kinase-like protein polynucleotide having a coding sequence disclosed herein and a polynucleotide sequence which is at least about 50, 55, 60, 65, 70, preferably about 75, 90, 96, or 98% identical to that nucleotide sequence can be calculated, for example, using the equation of Bolton and McCarthy, *Proc. Natl. Acad. Sci. U.S.A.* *48*, 1390 (1962):

$T_m = 81.5°C - 16.6(\log_{10}[Na^+]) + 0.41(\%G + C) - 0.63(\%\text{formamide}) - 600/l)$,
where l = the length of the hybrid in basepairs.

Stringent wash conditions include, for example, 4X SSC at 65°C, or 50% formamide, 4X SSC at 42°C, or 0.5X SSC, 0.1% SDS at 65°C. Highly stringent wash conditions include, for example, 0.2X SSC at 65°C.

Preparation of Polynucleotides

A naturally occurring sphingosine kinase-like protein polynucleotide can be isolated free of other cellular components such as membrane components, proteins, and lipids. Polynucleotides can be made by a cell and isolated using standard nucleic acid purification techniques, synthesized using an amplification technique, such as the polymerase chain reaction (PCR), or synthesized using an automatic synthesizer. Methods for isolating polynucleotides are routine and are known in the art. Any such

technique for obtaining a polynucleotide can be used to obtain isolated sphingosine kinase-like protein polynucleotides. For example, restriction enzymes and probes can be used to isolate polynucleotide fragments which comprise sphingosine kinase-like protein nucleotide sequences. Isolated polynucleotides are in preparations which are free or at least 70, 80, or 90% free of other molecules.

Sphingosine kinase-like protein cDNA molecules can be made with standard molecular biology techniques, using sphingosine kinase-like protein mRNA as a template. Sphingosine kinase-like protein cDNA molecules can thereafter be replicated using molecular biology techniques known in the art and disclosed in manuals such as Sambrook *et al.* (1989). An amplification technique, such as PCR, can be used to obtain additional copies of sphingosine kinase-like protein polynucleotides, using either human genomic DNA or cDNA as a template.

Alternatively, synthetic chemistry techniques can be used to synthesize sphingosine kinase-like protein polynucleotides. The degeneracy of the genetic code allows alternate nucleotide sequences to be synthesized which will encode a sphingosine kinase-like protein polypeptide having, for example, the amino acid sequence shown in SEQ ID NOS: 2, 10, and 11 or a biologically active variant of that sequence.

Obtaining Full-Length Polynucleotides

The partial sequence of SEQ ID NOS: 1 and 9 or its complement can be used to identify the corresponding full length gene from which it was derived. The partial sequences can be nick-translated or end-labeled with ^{32}P using polynucleotide kinase using labeling methods known to those with skill in the art (BASIC METHODS IN MOLECULAR BIOLOGY, Davis *et al.*, eds., Elsevier Press, N.Y., 1986). A lambda library prepared from human tissue can be directly screened with the labeled sequences of interest or the library can be converted en masse to pBluescript (Stratagene Cloning Systems, La Jolla, Calif. 92037) to facilitate bacterial colony screening (see Sambrook *et al.*, 1989, pg. 1.20).

Both methods are well known in the art. Briefly, filters with bacterial colonies containing the library in pBluescript or bacterial lawns containing lambda plaques are denatured, and the DNA is fixed to the filters. The filters are hybridized with the labeled probe using hybridization conditions described by Davis *et al.*, 1986. The partial sequences, cloned into lambda or pBluescript, can be used as positive controls to assess background binding and to adjust the hybridization and washing stringencies necessary for accurate clone identification. The resulting auto-radiograms are compared to duplicate plates of colonies or plaques; each exposed spot corresponds to a positive colony or plaque. The colonies or plaques are selected and expanded, and the DNA is isolated from the colonies for further analysis and sequencing.

Positive cDNA clones are analyzed to determine the amount of additional sequence they contain using PCR with one primer from the partial sequence and the other primer from the vector. Clones with a larger vector-insert PCR product than the original partial sequence are analyzed by restriction digestion and DNA sequencing to determine whether they contain an insert of the same size or similar as the mRNA size determined from Northern blot Analysis.

Once one or more overlapping cDNA clones are identified, the complete sequence of the clones can be determined, for example after exonuclease III digestion (McCombie *et al.*, *Methods 3*, 33-40, 1991). A series of deletion clones are generated, each of which is sequenced. The resulting overlapping sequences are assembled into a single contiguous sequence of high redundancy (usually three to five overlapping sequences at each nucleotide position), resulting in a highly accurate final sequence.

Various PCR-based methods can be used to extend the nucleic acid sequences encoding the disclosed portions of human sphingosine kinase-like protein to detect upstream sequences such as promoters and regulatory elements. For example,

restriction-site PCR uses universal primers to retrieve unknown sequence adjacent to a known locus (Sarkar, *PCR Methods Applic. 2*, 318-322, 1993). Genomic DNA is first amplified in the presence of a primer to a linker sequence and a primer specific to the known region. The amplified sequences are then subjected to a second round of PCR with the same linker primer and another specific primer internal to the first one. Products of each round of PCR are transcribed with an appropriate RNA polymerase and sequenced using reverse transcriptase.

Inverse PCR also can be used to amplify or extend sequences using divergent primers based on a known region (Triglia *et al.*, *Nucleic Acids Res. 16*, 8186, 1988). Primers can be designed using commercially available software, such as OLIGO 4.06 Primer Analysis software (National Biosciences Inc., Plymouth, Minn.), to be 22-30 nucleotides in length, to have a GC content of 50% or more, and to anneal to the target sequence at temperatures about 68 - 72°C. The method uses several restriction enzymes to generate a suitable fragment in the known region of a gene. The fragment is then circularized by intramolecular ligation and used as a PCR template.

Another method which can be used is capture PCR, which involves PCR amplification of DNA fragments adjacent to a known sequence in human and yeast artificial chromosome DNA (Lagerstrom *et al.*, *PCR Methods Applic. 1*, 111-119, 1991). In this method, multiple restriction enzyme digestions and ligations are used to place an engineered double-stranded sequence into an unknown fragment of the DNA molecule before performing PCR.

Another method which can be used to retrieve unknown sequences is that of Parker *et al.*, *Nucleic Acids Res. 19*, 3055-3060, 1991. Additionally, PCR, nested primers, and PROMOTERFINDER libraries (CLONTECH, Palo Alto, Calif.) can be used to walk genomic DNA. This process avoids the need to screen libraries and is useful in finding intron/exon junctions.

When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. Also, random-primed libraries are preferable, in that they will contain more sequences which contain the 5' regions of genes. Use of a randomly primed library may be especially preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries can be useful for extension of sequence into 5' non-transcribed regulatory regions.

Commercially available capillary electrophoresis systems can be used to analyze the size or confirm the nucleotide sequence of PCR or sequencing products. For example, capillary sequencing can employ flowable polymers for electrophoretic separation, four different fluorescent dyes (one for each nucleotide) which are laser activated, and detection of the emitted wavelengths by a charge coupled device camera. Output/light intensity can be converted to electrical signal using appropriate software (*e.g.* GENOTYPER and Sequence NAVIGATOR, Perkin Elmer), and the entire process from loading of samples to computer analysis and electronic data display can be computer controlled. Capillary electrophoresis is especially preferable for the sequencing of small pieces of DNA which might be present in limited amounts in a particular sample.

Obtaining Polypeptides

Sphingosine kinase-like protein polypeptides can be obtained, for example, by purification from human cells, by expression of sphingosine kinase-like protein polynucleotides, or by direct chemical synthesis.

Protein Purification

Sphingosine kinase-like protein polypeptides can be purified from human cells, such as primary tumor cells, metastatic cells, or cancer cell lines (*e.g.*, colon cancer cell lines HCT116, DLD1, HT29, Caco2, SW837, SW480, and RKO, breast cancer cell lines 21-PT, 21-MT, MDA-468, SK-BR3, and BT-474, the A549 lung cancer cell

line, or the H392 glioblastoma cell line). Carcinoma of the lung is an especially useful source of sphingosine kinase-like protein polypeptides. A purified sphingosine kinase-like protein polypeptide is separated from other compounds which normally associate with the sphingosine kinase-like protein polypeptide in the cell, such as certain proteins, carbohydrates, or lipids, using methods well-known in the art. Such methods include, but are not limited to, size exclusion chromatography, ammonium sulfate fractionation, ion exchange chromatography, affinity chromatography, and preparative gel electrophoresis. A preparation of purified sphingosine kinase-like protein polypeptides is at least 80% pure; preferably, the preparations are 90%, 95%, or 99% pure. Purity of the preparations can be assessed by any means known in the art, such as SDS-polyacrylamide gel electrophoresis. Enzymatic activity of the purified preparations can be assayed, for example, as described in Lin *et al.*, *J. Biol. Chem. 274*, 18231-36, 1999.

Expression of Polynucleotides

To express a sphingosine kinase-like protein polypeptide, a sphingosine kinase-like protein polynucleotide can be inserted into an expression vector which contains the necessary elements for the transcription and translation of the inserted coding sequence. Methods which are well known to those skilled in the art can be used to construct expression vectors containing sequences encoding sphingosine kinase-like protein polypeptides and appropriate transcriptional and translational control elements. These methods include *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. Such techniques are described, for example, in Sambrook *et al.* (1989) and Ausubel *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, N.Y, 1989.

A variety of expression vector/host systems can be utilized to contain and express sequences encoding a sphingosine kinase-like protein polypeptide. These include, but are not limited to, microorganisms, such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast

transformed with yeast expression vectors, insect cell systems infected with virus expression vectors (*e.g.*, baculovirus), plant cell systems transformed with virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or with bacterial expression vectors (*e.g.*, Ti or pBR322 plasmids), or animal cell systems.

The control elements or regulatory sequences are those non-translated regions of the vector -- enhancers, promoters, 5' and 3' untranslated regions -- which interact with host cellular proteins to carry out transcription and translation. Such elements can vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible promoters, can be used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid lacZ promoter of the BLUESCRIPT phagemid (Stratagene, LaJolla, Calif.) or pSPORT1 plasmid (Life Technologies) and the like can be used. The baculovirus polyhedrin promoter can be used in insect cells. Promoters or enhancers derived from the genomes of plant cells (*e.g.*, heat shock, RUBISCO, and storage protein genes) or from plant viruses (*e.g.*, viral promoters or leader sequences) can be cloned into the vector. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are preferable. If it is necessary to generate a cell line that contains multiple copies of a nucleotide sequence encoding a sphingosine kinase-like protein polypeptide, vectors based on SV40 or EBV can be used with an appropriate selectable marker.

Bacterial and Yeast Expression Systems

In bacterial systems, a number of expression vectors can be selected depending upon the use intended for the sphingosine kinase-like protein polypeptide. For example, when a large quantity of a sphingosine kinase-like protein polypeptide is needed for the induction of antibodies, vectors which direct high level expression of fusion proteins that are readily purified can be used. Such vectors include, but are not limited to, multifunctional *E. coli* cloning and expression vectors such as

BLUESCRIPT (Stratagene), in which the sequence encoding the sphingosine kinase-like protein polypeptide can be ligated into the vector in frame with sequences for the amino-terminal Met and the subsequent 7 residues of β-galactosidase so that a hybrid protein is produced. pIN vectors (Van Heeke & Schuster, *J. Biol. Chem. 264*, 5503-5509, 1989 or pGEX vectors (Promega, Madison, Wis.) can be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. Proteins made in such systems can be designed to include heparin, thrombin, or Factor Xa protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety at will.

In the yeast *Saccharomyces cerevisiae*, a number of vectors containing constitutive or inducible promoters such as alpha factor, alcohol oxidase, and PGH can be used. For reviews, see Ausubel *et al.* (1989) and Grant *et al.*, *Methods Enzymol. 153*, 516-544, 1987.

Plant and Insect Expression Systems

If plant expression vectors are used, the expression of sequences encoding sphingosine kinase-like protein polypeptides can be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of CaMV can be used alone or in combination with the omega leader sequence from TMV (Takamatsu *EMBO J. 6*, 307-311, 1987). Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters can be used (Coruzzi *et al.*, *EMBO J. 3*, 1671-1680, 1984; Broglie *et al.*, *Science 224*, 838-843, 1984; Winter *et al.*, *Results Probl. Cell Differ. 17*, 85-105, 1991). These constructs can be introduced into plant cells by direct DNA transformation or by pathogen-mediated transfection. Such techniques are described in a number of generally available reviews (see, for example, Hobbs or Murray, in MCGRAW HILL YEARBOOK OF SCIENCE AND TECHNOLOGY, McGraw Hill, New York, N.Y., pp. 191-196, 1992).

An insect system also can be used to express a sphingosine kinase-like protein polypeptide. For example, in one such system *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in *Spodoptera frugiperda* cells or in *Trichoplusia* larvae. Sequences encoding sphingosine kinase-like protein polypeptides can be cloned into a non-essential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of sphingosine kinase-like protein polypeptides will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein. The recombinant viruses can then be used to infect, for example, *S. frugiperda cells* or *Trichoplusia* larvae in which sphingosine kinase-like protein polypeptides can be expressed (Engelhard *et al.*, *Proc. Nat. Acad. Sci. 91*, 3224-3227, 1994).

Mammalian Expression Systems

A number of viral-based expression systems can be utilized in mammalian host cells. For example, if an adenovirus is used as an expression vector, sequences encoding sphingosine kinase-like protein polypeptides can be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome can be used to obtain a viable virus which is capable of expressing a sphingosine kinase-like protein polypeptide in infected host cells (Logan & Shenk, *Proc. Natl. Acad. Sci. 81*, 3655-3659, 1984). In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, can be used to increase expression in mammalian host cells.

Human artificial chromosomes (HACs) also can be used to deliver larger fragments of DNA than can be contained and expressed in a plasmid. HACs of 6M to 10M are constructed and delivered to cells via conventional delivery methods (*e.g.*, liposomes, polycationic amino polymers, or vesicles).

Specific initiation signals also can be used to achieve more efficient translation of sequences encoding sphingosine kinase-like protein polypeptides. Such signals

include the ATG initiation codon and adjacent sequences. In cases where sequences encoding a sphingosine kinase-like protein polypeptide, its initiation codon, and upstream sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a fragment thereof, is inserted, exogenous translational control signals (including the ATG initiation codon) should be provided. The initiation codon should be in the correct reading frame to ensure translation of the entire insert. Exogenous translational elements and initiation codons can be of various origins, both natural and synthetic. The efficiency of expression can be enhanced by the inclusion of enhancers which are appropriate for the particular cell system which is used (see Scharf *et al.*, *Results Probl. Cell Differ. 20*, 125-162, 1994).

Host Cells

A host cell strain can be chosen for its ability to modulate the expression of the inserted sequences or to process an expressed sphingosine kinase-like protein polypeptide in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" form of the polypeptide also can be used to facilitate correct insertion, folding and/or function. Different host cells which have specific cellular machinery and characteristic mechanisms for post-translational activities (*e.g.*, CHO, HeLa, MDCK, HEK293, and WI38), are available from the American Type Culture Collection (ATCC; 10801 University Boulevard, Manassas, VA 20110-2209) and can be chosen to ensure the correct modification and processing of the foreign protein.

Stable expression is preferred for long-term, high-yield production of recombinant proteins. For example, cell lines which stably express sphingosine kinase-like protein polypeptides can be transformed using expression vectors which can contain viral origins of replication and/or endogenous expression elements and a selectable

marker gene on the same or on a separate vector. Following the introduction of the vector, cells can be allowed to grow for 1-2 days in an enriched medium before they are switched to a selective medium. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced sphingosine kinase-like protein sequences. Resistant clones of stably transformed cells can be proliferated using tissue culture techniques appropriate to the cell type.

Any number of selection systems can be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase (Wigler *et al.*, *Cell 11*, 223-32, 1977) and adenine phosphoribosyltransferase (Lowy *et al.*, *Cell 22*, 817-23, 1980). Genes which can be employed in *tk*$^-$ or *aprt*$^-$ cells, respectively. Also, antimetabolite, antibiotic, or herbicide resistance can be used as the basis for selection. For example, *dhfr* confers resistance to methotrexate (Wigler *et al.*, *Proc. Natl. Acad. Sci. 77*, 3567-70, 1980); *npt* confers resistance to the aminoglycosides, neomycin and G-418 (Colbere-Garapin *et al.*, *J. Mol. Biol. 150*, 1-14, 1981); and *als* and *pat* confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively (Murray, 1992 *supra*). Additional selectable genes have been described, for example *trpB*, which allows cells to utilize indole in place of tryptophan, or *hisD*, which allows cells to utilize histinol in place of histidine (Hartman & Mulligan, *Proc. Natl. Acad. Sci. 85*, 8047-51, 1988). Visible markers such as anthocyanins, β-glucuronidase and its substrate GUS, and luciferase and its substrate luciferin, can be used to identify transformants and to quantify the amount of transient or stable protein expression attributable to a specific vector system (Rhodes *et al.*, *Methods Mol. Biol. 55*, 121-131, 1995).

Detecting Expression of Polypeptides

Although the presence of marker gene expression suggests that the sphingosine kinase-like protein polynucleotide is also present, its presence and expression may need to be confirmed. For example, if a sequence encoding a sphingosine kinase-like

protein polypeptide is inserted within a marker gene sequence, transformed cells containing sequences which encode a sphingosine kinase-like protein polypeptide can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding a sphingosine kinase-like protein polypeptide under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the sphingosine kinase-like protein polynucleotide.

Alternatively, host cells which contain a sphingosine kinase-like protein polynucleotide and which express a sphingosine kinase-like protein polypeptide can be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations and protein bioassay or immunoassay techniques which include membrane, solution, or chip-based technologies for the detection and/or quantification of nucleic acid or protein.

The presence of a polynucleotide sequence encoding a sphingosine kinase-like protein polypeptide can be detected by DNA-DNA or DNA-RNA hybridization or amplification using probes or fragments or fragments of polynucleotides encoding a sphingosine kinase-like protein polypeptide. Nucleic acid amplification-based assays involve the use of oligonucleotides selected from sequences encoding a sphingosine kinase-like protein polypeptide to detect transformants which contain a sphingosine kinase-like protein polynucleotide.

A variety of protocols for detecting and measuring the expression of a sphingosine kinase-like protein polypeptide, using either polyclonal or monoclonal antibodies specific for the polypeptide, are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay using monoclonal antibodies reactive to two non-interfering epitopes on a sphingosine kinase-like protein polypeptide can be used, or a competitive binding assay can be employed.

These and other assays are described in Hampton *et al.*, SEROLOGICAL METHODS: A LABORATORY MANUAL, APS Press, St. Paul, Minn., 1990) and Maddox *et al.*, *J. Exp. Med. 158*, 1211-1216, 1983).

A wide variety of labels and conjugation techniques are known by those skilled in the art and can be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides encoding sphingosine kinase-like protein polypeptides include oligolabeling, nick translation, end-labeling, or PCR amplification using a labeled nucleotide. Alternatively, sequences encoding a sphingosine kinase-like protein polypeptide can be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and can be used to synthesize RNA probes *in vitro* by addition of labeled nucleotides and an appropriate RNA polymerase, such as T7, T3, or SP6. These procedures can be conducted using a variety of commercially available kits (Amersham Pharmacia Biotech, Promega, and US Biochemical). Suitable reporter molecules or labels which can be used for ease of detection include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents, as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Expression and Purification of Polypeptides

Host cells transformed with nucleotide sequences encoding a sphingosine kinase-like protein polypeptide can be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The polypeptide produced by a transformed cell can be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode sphingosine kinase-like protein polypeptides can be designed to contain signal sequences which direct secretion of sphingosine kinase-like protein polypeptides through a prokaryotic or eukaryotic cell membrane.

Other constructions can be used to join a sequence encoding a sphingosine kinase-like protein polypeptide to a nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp., Seattle, Wash.). The inclusion of cleavable linker sequences such as those specific for Factor Xa or enterokinase (Invitrogen, San Diego, CA) between the purification domain and the sphingosine kinase-like protein polypeptide can be used to facilitate purification. One such expression vector provides for expression of a fusion protein containing a sphingosine kinase-like protein polypeptide and 6 histidine residues preceding a thioredoxin or an enterokinase cleavage site. The histidine residues facilitate purification on IMAC (immobilized metal ion affinity chromatography as described in Porath *et al.*, *Prot. Exp. Purif. 3*, 263-281, 1992), while the enterokinase cleavage site provides a means for purifying the sphingosine kinase-like protein polypeptide from the fusion protein. Vectors which contain fusion proteins are disclosed in Kroll *et al.*, *DNA Cell Biol. 12*, 441-453, 1993).

Chemical Synthesis

Sequences encoding a sphingosine kinase-like protein polypeptide can be synthesized, in whole or in part, using chemical methods well known in the art (see Caruthers *et al.*, *Nucl. Acids Res. Symp. Ser.* 215-223, 1980; Horn *et al. Nucl. Acids Res. Symp. Ser.* 225-232, 1980). Alternatively, a sphingosine kinase-like protein polypeptide itself can be produced using chemical methods to synthesize its amino acid sequence. For example, sphingosine kinase-like protein polypeptides can be produced by direct peptide synthesis using solid-phase techniques (Merrifield, *J. Am. Chem. Soc. 85*, 2149-2154, 1963; Roberge *et al.*, *Science 269*, 202-204, 1995). Protein synthesis can be performed using manual techniques or by automation.

Automated synthesis can be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer). Various fragments of sphingosine kinase-like protein polypeptides can be separately synthesized and combined using chemical methods to produce a full-length molecule.

The newly synthesized peptide can be substantially purified by preparative high performance liquid chromatography (*e.g.*, Creighton, PROTEINS: STRUCTURES AND MOLECULAR PRINCIPLES, WH Freeman and Co., New York, N.Y., 1983). The composition of a synthetic sphingosine kinase-like protein polypeptide can be confirmed by amino acid analysis or sequencing (*e.g.*, the Edman degradation procedure; *see* Creighton, *supra*). Additionally, any portion of the amino acid sequence of the sphingosine kinase-like protein polypeptide can be altered during direct synthesis and/or combined using chemical methods with sequences from other proteins to produce a variant polypeptide or a fusion protein.

Production of Altered Polypeptides

As will be understood by those of skill in the art, it may be advantageous to produce sphingosine kinase-like protein polypeptide-encoding nucleotide sequences possessing non-naturally occurring codons. For example, codons preferred by a particular prokaryotic or eukaryotic host can be selected to increase the rate of protein expression or to produce an RNA transcript having desirable properties, such as a half-life which is longer than that of a transcript generated from the naturally occurring sequence.

The nucleotide sequences disclosed herein can be engineered using methods generally known in the art to alter sphingosine kinase-like protein polypeptide-encoding sequences for a variety of reasons, including modification of the cloning, processing, and/or expression of the gene product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides can be used to engineer the nucleotide sequences. For example, site-directed

mutagenesis can be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, introduce mutations, and so forth.

Antibodies

Any type of antibody known in the art can be generated to bind specifically to an epitope of a sphingosine kinase-like protein polypeptide. "Antibody" as used herein includes intact immunoglobulin molecules, as well as fragments thereof, such as Fab, $F(ab')_2$, and Fv, which are capable of binding an epitope of a sphingosine kinase-like protein polypeptide. Typically, at least 6, 8, 10, or 12 contiguous amino acids are required to form an epitope. However, epitopes which involve non-contiguous amino acids may require more, *e.g.*, at least 15, 25, or 50 amino acids.

An antibody which specifically binds to an epitope of a sphingosine kinase-like protein polypeptide can be used therapeutically, as well as in immunochemical assays, including but not limited to Western blots, ELISAs, radioimmunoassays, immunohistochemical assays, immunoprecipitations, or other immunochemical assays known in the art. Various immunoassays can be used to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays are well known in the art. Such immunoassays typically involve the measurement of complex formation between an immunogen and an antibody which specifically binds to the immunogen.

Typically, an antibody which specifically binds to a sphingosine kinase-like protein polypeptide provides a detection signal at least 5-, 10-, or 20-fold higher than a detection signal provided with other proteins when used in an immunochemical assay. Preferably, antibodies which specifically bind to sphingosine kinase-like protein polypeptides do not detect other proteins in immunochemical assays and can immunoprecipitate a sphingosine kinase-like protein polypeptide from solution.

Sphingosine kinase-like protein polypeptides can be used to immunize a mammal, such as a mouse, rat, rabbit, guinea pig, monkey, or human, to produce polyclonal antibodies. If desired, a sphingosine kinase-like protein polypeptide can be conjugated to a carrier protein, such as bovine serum albumin, thyroglobulin, and keyhole limpet hemocyanin. Depending on the host species, various adjuvants can be used to increase the immunological response. Such adjuvants include, but are not limited to, Freund's adjuvant, mineral gels (*e.g.*, aluminum hydroxide), and surface active substances (*e.g.* lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol). Among adjuvants used in humans, BCG (*bacilli Calmette-Guerin*) and *Corynebacterium parvum* are especially useful.

Monoclonal antibodies which specifically bind to a sphingosine kinase-like protein polypeptide can be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These techniques include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique (Kohler *et al.*, *Nature 256*, 495-497, 1985; Kozbor *et al.*, *J. Immunol. Methods 81*, 31-42, 1985; Cote *et al.*, *Proc. Natl. Acad. Sci. 80*, 2026-2030, 1983; Cole *et al.*, *Mol. Cell Biol. 62*, 109-120, 1984).

In addition, techniques developed for the production of "chimeric antibodies," the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity, can be used (Morrison *et al.*, *Proc. Natl. Acad. Sci. 81*, 6851-6855, 1984; Neuberger *et al.*, *Nature 312*, 604-608, 1984; Takeda *et al.*, *Nature 314*, 452-454, 1985). Monoclonal and other antibodies also can be "humanized" to prevent a patient from mounting an immune response against the antibody when it is used therapeutically. Such antibodies may be sufficiently similar in sequence to human antibodies to be used directly in therapy or may require alteration of a few key residues. Sequence differences between rodent antibodies and human sequences can be minimized by replacing residues which differ from those in the human sequences by site directed mutagenesis of individual

residues or by grating of entire complementarity determining regions. Alternatively, one can produce humanized antibodies using recombinant methods, as described in GB2188638B. Antibodies which specifically bind to a sphingosine kinase-like protein polypeptide can contain antigen binding sites which are either partially or fully humanized, as disclosed in U.S. 5,565,332.

Alternatively, techniques described for the production of single chain antibodies can be adapted using methods known in the art to produce single chain antibodies which specifically bind to sphingosine kinase-like protein polypeptides. Antibodies with related specificity, but of distinct idiotypic composition, can be generated by chain shuffling from random combinatorial immunoglobin libraries (Burton, *Proc. Natl. Acad. Sci. 88*, 11120-23, 1991).

Single-chain antibodies also can be constructed using a DNA amplification method, such as PCR, using hybridoma cDNA as a template (Thirion *et al.*, 1996, *Eur. J. Cancer Prev. 5*, 507-11). Single-chain antibodies can be mono- or bispecific, and can be bivalent or tetravalent. Construction of tetravalent, bispecific single-chain antibodies is taught, for example, in Coloma & Morrison, 1997, *Nat. Biotechnol. 15*, 159-63. Construction of bivalent, bispecific single-chain antibodies is taught in Mallender & Voss, 1994, *J. Biol. Chem. 269*, 199-206.

A nucleotide sequence encoding a single-chain antibody can be constructed using manual or automated nucleotide synthesis, cloned into an expression construct using standard recombinant DNA methods, and introduced into a cell to express the coding sequence, as described below. Alternatively, single-chain antibodies can be produced directly using, for example, filamentous phage technology. Verhaar *et al.*, 1995, *Int. J. Cancer 61*, 497-501; Nicholls *et al.*, 1993, *J. Immunol. Meth. 165*, 81-91.

Antibodies which specifically bind to sphingosine kinase-like protein polypeptides also can be produced by inducing *in vivo* production in the lymphocyte population or

by screening immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature (Orlandi *et al.*, *Proc. Natl. Acad. Sci. 86*, 3833-3837, 1989; Winter *et al.*, *Nature 349*, 293-299, 1991).

Other types of antibodies can be constructed and used therapeutically in methods of the invention. For example, chimeric antibodies can be constructed as disclosed in WO 93/03151. Binding proteins which are derived from immunoglobulins and which are multivalent and multispecific, such as the "diabodies" described in WO 94/13804, also can be prepared.

Antibodies of the invention can be purified by methods well known in the art. For example, antibodies can be affinity purified by passage over a column to which a sphingosine kinase-like protein polypeptide is bound. The bound antibodies can then be eluted from the column using a buffer with a high salt concentration.

Antisense Oligonucleotides

Antisense oligonucleotides are nucleotide sequences which are complementary to a specific DNA or RNA sequence. Once introduced into a cell, the complementary nucleotides combine with natural sequences produced by the cell to form complexes and block either transcription or translation. Preferably, an antisense oligonucleotide is at least 11 nucleotides in length, but can be at least 12, 15, 20, 25, 30, 35, 40, 45, or 50 or more nucleotides long. Longer sequences also can be used. Antisense oligonucleotide molecules can be provided in a DNA construct and introduced into a cell as described above to decrease the level of sphingosine kinase-like protein gene products in the cell.

Antisense oligonucleotides can be deoxyribonucleotides, ribonucleotides, or a combination of both. Oligonucleotides can be synthesized manually or by an automated synthesizer, by covalently linking the 5' end of one nucleotide with the 3' end of another nucleotide with non-phosphodiester internucleotide linkages such alkyl-

phosphonates, phosphorothioates, phosphorodithioates, alkylphosphonothioates, alkylphosphonates, phosphoramidates, phosphate esters, carbamates, acetamidate, carboxymethyl esters, carbonates, and phosphate triesters. *See* Brown, *Meth. Mol. Biol. 20*, 1-8, 1994; Sonveaux, *Meth. Mol. Biol. 26*, 1-72, 1994; Uhlmann *et al.*, *Chem. Rev. 90*, 543-583, 1990.

Modifications of sphingosine kinase-like protein gene expression can be obtained by designing antisense oligonucleotides which will form duplexes to the control, 5', or regulatory regions of the sphingosine kinase-like protein gene. Oligonucleotides derived from the transcription initiation site, *e.g.*, between positions -10 and +10 from the start site, are preferred. Similarly, inhibition can be achieved using "triple helix" base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or chaperons. Therapeutic advances using triplex DNA have been described in the literature (*e.g.*, Gee *et al.*, in Huber & Carr, MOLECULAR AND IMMUNOLOGIC APPROACHES, Futura Publishing Co., Mt. Kisco, N.Y., 1994). An antisense oligonucleotide also can be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

Precise complementarity is not required for successful duplex formation between an antisense oligonucleotide and the complementary sequence of a sphingosine kinase-like protein polynucleotide. Antisense oligonucleotides which comprise, for example, 2, 3, 4, or 5 or more stretches of contiguous nucleotides which are precisely complementary to a sphingosine kinase-like protein polynucleotide, each separated by a stretch of contiguous nucleotides which are not complementary to adjacent sphingosine kinase-like protein nucleotides, can provide targeting specificity for sphingosine kinase-like protein mRNA. Preferably, each stretch of complementary contiguous nucleotides is at least 4, 5, 6, 7, or 8 or more nucleotides in length. Non-complementary intervening sequences are preferably 1, 2, 3, or 4 nucleotides in length. One skilled in the art can easily use the calculated melting point of an antisense-sense pair to determine the degree of mismatching which will be tolerated

between a particular antisense oligonucleotide and a particular sphingosine kinase-like protein polynucleotide sequence.

Antisense oligonucleotides can be modified without affecting their ability to hybridize to a sphingosine kinase-like protein polynucleotide. These modifications can be internal or at one or both ends of the antisense molecule. For example, internucleoside phosphate linkages can be modified by adding cholesteryl or diamine moieties with varying numbers of carbon residues between the amino groups and terminal ribose. Modified bases and/or sugars, such as arabinose instead of ribose, or a 3', 5'-substituted oligonucleotide in which the 3' hydroxyl group or the 5' phosphate group are substituted, also can be employed in a modified antisense oligonucleotide. These modified oligonucleotides can be prepared by methods well known in the art. *See, e.g.*, Agrawal *et al.*, *Trends Biotechnol. 10*, 152-158, 1992; Uhlmann *et al.*, *Chem. Rev. 90*, 543-584, 1990; Uhlmann *et al.*, *Tetrahedron. Lett. 215*, 3539-3542, 1987.

Ribozymes

Ribozymes are RNA molecules with catalytic activity. *See, e.g.*, Cech, *Science 236*, 1532-1539; 1987; Cech, *Ann. Rev. Biochem. 59*, 543-568; 1990, Cech, *Curr. Opin. Struct. Biol. 2*, 605-609; 1992, Couture & Stinchcomb, *Trends Genet. 12*, 510-515, 1996. Ribozymes can be used to inhibit gene function by cleaving an RNA sequence, as is known in the art (*e.g.*, Haseloff *et al.*, U.S. Patent 5,641,673). The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Examples include engineered hammerhead motif ribozyme molecules that can specifically and efficiently catalyze endonucleolytic cleavage of specific nucleotide sequences.

The coding sequence of a sphingosine kinase-like protein polynucleotide can be used to generate ribozymes which will specifically bind to mRNA transcribed from the

sphingosine kinase-like protein polynucleotide. Methods of designing and constructing ribozymes which can cleave other RNA molecules in trans in a highly sequence specific manner have been developed and described in the art (*see* Haseloff *et al. Nature 334*, 585-591, 1988). For example, the cleavage activity of ribozymes can be targeted to specific RNAs by engineering a discrete "hybridization" region into the ribozyme. The hybridization region contains a sequence complementary to the target RNA and thus specifically hybridizes with the target (see, for example, Gerlach *et al.*, EP 321,201).

Specific ribozyme cleavage sites within a sphingosine kinase-like protein RNA target are initially identified by scanning the RNA molecule for ribozyme cleavage sites which include the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the sphingosine kinase-like protein target RNA containing the cleavage site can be evaluated for secondary structural features which may render the target inoperable. The suitability of candidate targets also can be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays. Longer complementary sequences can be used to increase the affinity of the hybridization sequence for the target. The hybridizing and cleavage regions of the ribozyme can be integrally related; thus, upon hybridizing to the sphingosine kinase-like protein target RNA through the complementary regions, the catalytic region of the ribozyme can cleave the target.

Ribozymes can be introduced into cells as part of a DNA construct. Mechanical methods, such as microinjection, liposome-mediated transfection, electroporation, or calcium phosphate precipitation, can be used to introduce a ribozyme-containing DNA construct into cells in which it is desired to decrease sphingosine kinase-like protein expression. Alternatively, if it is desired that the cells stably retain the DNA construct, it can be supplied on a plasmid and maintained as a separate element or integrated into the genome of the cells, as is known in the art. The DNA construct can include transcriptional regulatory elements, such as a promoter element, an

enhancer or UAS element, and a transcriptional terminator signal, for controlling transcription of ribozymes in the cells.

As taught in Haseloff *et al.*, U.S. Patent 5,641,673, ribozymes can be engineered so that ribozyme expression will occur in response to factors which induce expression of a target gene. Ribozymes also can be engineered to provide an additional level of regulation, so that destruction of sphingosine kinase-like protein mRNA occurs only when both a ribozyme and a target gene are induced in the cells.

Differentially Expressed Genes

Described herein are methods for the identification of genes whose products interact with human sphingosine kinase-like protein. Such genes may represent genes which are differentially expressed in disorders including, but not limited to, cancer, allergies including but not limited to asthma, central and peripheral nervous system disorders, and autoimmune disease. Further, such genes may represent genes which are differentially regulated in response to manipulations relevant to the progression or treatment of such diseases. Additionally, such genes may have a temporally modulated expression, increased or decreased at different stages of tissue or organism development. A differentially expressed gene may also have its expression modulated under control versus experimental conditions. In addition, the human sphingosine kinase-like gene or gene product may itself be tested for differential expression.

The degree to which expression differs in a normal versus a diseased state need only be large enough to be visualized via standard characterization techniques such as differential display techniques. Other such standard characterization techniques by which expression differences may be visualized include but are not limited to, quantitative RT (reverse transcriptase), PCR, and Northern analysis.

Identification of Differentially Expressed Genes

To identify differentially expressed genes total RNA or, preferably, mRNA is isolated from tissues of interest. For example, RNA samples are obtained from tissues of experimental subjects and from corresponding tissues of control subjects. Any RNA isolation technique which does not select against the isolation of mRNA may be utilized for the purification of such RNA samples. See, for example, Ausubel *et al.*, ed.,, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, Inc. New York, 1987-1993. Large numbers of tissue samples may readily be processed using techniques well known to those of skill in the art, such as, for example, the single-step RNA isolation process of Chomczynski, U.S. Patent 4,843,155.

Transcripts within the collected RNA samples which represent RNA produced by differentially expressed genes are identified by methods well known to those of skill in the art. They include, for example, differential screening (Tedder *et al., Proc. Natl. Acad. Sci. U.S.A. 85*, 208-12, 1988), subtractive hybridization (Hedrick *et al., Nature 308*, 149-53; Lee *et al., Proc. Natl. Acad. Sci. U.S.A. 88*, 2825, 1984), and, preferably, differential display (Liang & Pardee, *Science 257*, 967-71, 1992; U.S. Patent 5,262,311).

The differential expression information may itself suggest relevant methods for the treatment of disorders involving the human sphingosine kinase-like protein. For example, treatment may include a modulation of expression of the differentially expressed genes and/or the gene encoding the human sphingosine kinase-like protein. The differential expression information may indicate whether the expression or activity of the differentially expressed gene or gene product or the human sphingosine kinase-like gene or gene product are up-regulated or down-regulated.

Screening Methods

The invention provides methods for identifying modulators, *i.e.*, candidate or test compounds which bind to sphingosine kinase-like protein polypeptides or polynucleotides and/or have a stimulatory or inhibitory effect on, for example, expression or activity of the sphingosine kinase-like protein polypeptide or polynucleotide, so as to regulate degradation of the polyamines. Decreased intracellular signaling is useful for preventing or suppressing malignant cells from metastasizing. Increased intracellular signaling may be desired, for example, in developmental disorders characterized by inappropriately low levels of intracellular signaling or in regeneration.

The invention provides assays for screening test compounds which bind to or modulate the activity of a sphingosine kinase-like protein polypeptide or a sphingosine kinase-like protein polynucleotide. A test compound preferably binds to a sphingosine kinase-like protein polypeptide or polynucleotide. More preferably, a test compound decreases a sphingosine kinase-like protein activity of a sphingosine kinase-like protein polypeptide or expression of a sphingosine kinase-like protein polynucleotide by at least about 10, preferably about 50, more preferably about 75, 90, or 100% relative to the absence of the test compound.

Test Compounds

Test compounds can be pharmacologic agents already known in the art or can be compounds previously unknown to have any pharmacological activity. Such compounds also may include, but are not limited to, other cellular proteins, peptides such as, for example, soluble peptides, including but not limited to, Ig-tailed fusion peptides, comprising extracellular portions of target gene product transmembrane receptors, and members of random peptide libraries (Lam, *et al.*, *Nature 354*, 82-84, 1991; Houghten *et al.*, *Nature 354*, 84-86, 1991), made of D- and/or L-configuration amino acids, phosphopeptides (including, but not limited to members of random or

partially degenerate, directed phosphopeptide libraries (Songyang *et al.*, *Cell 72*, 767-78, 1993), antibodies (including, but not limited to, polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain antibodies, and Fab, $F(ab')_2$ and Fab expression library fragments, and epitope-binding fragments thereof), and small organic or inorganic molecules.

The compounds can be naturally occurring or designed in the laboratory. They can be isolated from microorganisms, animals, or plants, and can be produced recombinantly, or synthesized by chemical methods known in the art. If desired, test compounds can be obtained using any of the numerous combinatorial library methods known in the art, including but not limited to, biological libraries, spatially addressable parallel solid phase or solution phase libraries, synthetic library methods requiring deconvolution, the "one-bead one-compound" library method, and synthetic library methods using affinity chromatography selection. The biological library approach is limited to polypeptide libraries, while the other four approaches are applicable to polypeptide, non-peptide oligomer, or small molecule libraries of compounds. *See* Lam, *Anticancer Drug Des. 12*, 145, 1997.

Methods for the synthesis of molecular libraries are well known in the art (*see*, for example, DeWitt *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 90*, 6909, 1993; Erb *et al. Proc. Natl. Acad. Sci. U.S.A. 91*, 11422, 1994; Zuckermann *et al.*, *J. Med. Chem. 37*, 2678, 1994; Cho *et al.*, *Science 261*, 1303, 1993; Carell *et al.*, *Angew. Chem. Int. Ed. Engl. 33*, 2059, 1994; Carell *et al.*, *Angew. Chem. Int. Ed. Engl. 33*, 2061; Gallop *et al.*, *J. Med. Chem. 37*, 1233, 1994). Libraries of compounds can be presented in solution (*see, e.g.*, Houghten, *Biotechniques 13*, 412-421, 1992), or on beads (Lam, *Nature 354*, 82-84, 1991), chips (Fodor, *Nature 364*, 555-556, 1993), bacteria or spores (Ladner, U.S. Patent 5,223,409), plasmids (Cull *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 89*, 1865-1869, 1992), or phage (Scott & Smith, *Science 249*, 386-390, 1990; Devlin, *Science 249*, 404-406, 1990); Cwirla *et al.*, *Proc. Natl. Acad. Sci. 97*, 6378-6382, 1990; Felici, *J. Mol. Biol. 222*, 301-310, 1991; and Ladner, U.S. Patent 5,223,409).

High Throughput Screening

Test compounds can be screened for the ability to bind to sphingosine kinase-like protein polypeptides or polynucleotides or to affect sphingosine kinase-like protein activity or sphingosine kinase-like protein gene expression using high throughput screening. Using high throughput screening, many discrete compounds can be tested in parallel so that large numbers of test compounds can be quickly screened. The most widely established techniques utilize 96-well microtiter plates. The wells of the microtiter plates typically require assay volumes that range from 50 to 500 µl. In addition to the plates, many instruments, materials, pipettors, robotics, plate washers, and plate readers are commercially available to fit the 96-well format.

Alternatively, "free format assays," or assays that have no physical barrier between samples, can be used. For example, an assay using pigment cells (melanocytes) in a simple homogeneous assay for combinatorial peptide libraries is described by Jayawickreme *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 19,* 1614-18 (1994). The cells are placed under agarose in petri dishes, then beads that carry combinatorial compounds are placed on the surface of the agarose. The combinatorial compounds are partially released the compounds from the beads. Active compounds can be visualized as dark pigment areas because, as the compounds diffuse locally into the gel matrix, the active compounds cause the cells to change colors.

Another example of a free format assay is described by Chelsky, "Strategies for Screening Combinatorial Libraries: Novel and Traditional Approaches," reported at the First Annual Conference of The Society for Biomolecular Screening in Philadelphia, Pa. (Nov. 7-10, 1995). Chelsky placed a simple homogenous enzyme assay for carbonic anhydrase inside an agarose gel such that the enzyme in the gel would cause a color change throughout the gel. Thereafter, beads carrying combinatorial compounds via a photolinker were placed inside the gel and the compounds were partially released by UV-light. Compounds that inhibited the enzyme were observed as local zones of inhibition having less color change.

Yet another example is described by Salmon *et al.*, *Molecular Diversity 2*, 57-63 (1996). In this example, combinatorial libraries were screened for compounds that had cytotoxic effects on cancer cells growing in agar.

Another high throughput screening method is described in Beutel *et al.*, U.S. Patent 5,976,813. In this method, test samples are placed in a porous matrix. One or more assay components are then placed within, on top of, or at the bottom of a matrix such as a gel, a plastic sheet, a filter, or other form of easily manipulated solid support. When samples are introduced to the porous matrix they diffuse sufficiently slowly, such that the assays can be performed without the test samples running together.

Binding Assays

For binding assays, the test compound is preferably a small molecule which binds to and occupies the active site or the diacylglycerol kinase domain of the sphingosine kinase-like protein polypeptide, such that normal biological activity is prevented. Examples of such small molecules include, but are not limited to, small peptides or peptide-like molecules. In binding assays, either the test compound or the sphingosine kinase-like protein polypeptide can comprise a detectable label, such as a fluorescent, radioisotopic, chemiluminescent, or enzymatic label, such as horseradish peroxidase, alkaline phosphatase, or luciferase. Detection of a test compound which is bound to the sphingosine kinase-like protein polypeptide can then be accomplished, for example, by direct counting of radioemmission, by scintillation counting, or by determining conversion of an appropriate substrate to a detectable product.

Alternatively, binding of a test compound to a sphingosine kinase-like protein polypeptide can be determined without labeling either of the interactants. For example, a microphysiometer can be used to detect binding of a test compound with a target polypeptide. A microphysiometer (*e.g.*, Cytosensor™) is an analytical

instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between a test compound and a sphingosine kinase-like protein polypeptide. (McConnell *et al.*, *Science 257*, 1906-1912, 1992).

Determining the ability of a test compound to bind to a sphingosine kinase-like protein polypeptide also can be accomplished using a technology such as real-time Bimolecular Interaction Analysis (BIA). Sjolander & Urbaniczky, *Anal. Chem. 63*, 2338-2345, 1991, and Szabo *et al.*, *Curr. Opin. Struct. Biol. 5*, 699-705, 1995. BIA is a technology for studying biospecific interactions in real time, without labeling any of the interactants (*e.g.*, BIAcore™). Changes in the optical phenomenon surface plasmon resonance (SPR) can be used as an indication of real-time reactions between biological molecules.

In yet another aspect of the invention, a sphingosine kinase-like protein polypeptide can be used as a "bait protein" in a two-hybrid assay or three-hybrid assay (see, *e.g.*, U.S. Patent 5,283,317; Zervos *et al.*, *Cell 72*, 223-232, 1993; Madura *et al.*, *J. Biol. Chem. 268*, 12046-12054, 1993; Bartel *et al.*, *Biotechniques 14*, 920-924, 1993; Iwabuchi *et al.*, *Oncogene 8*, 1693-1696, 1993; and Brent W094/10300), to identify other proteins which bind to or interact with the sphingosine kinase-like protein polypeptide and modulate its activity.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. For example, in one construct a polynucleotide encoding a sphingosine kinase-like protein polypeptide is fused to a polynucleotide encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In the other construct, a DNA sequence that encodes an unidentified protein ("prey" or "sample") is fused to a polynucleotide that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are

able to interact *in vivo* to form an protein-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, LacZ), which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected, and cell colonies containing the functional transcription factor can be isolated and used to obtain the DNA sequence encoding the protein which interacts with the sphingosine kinase-like protein polypeptide.

It may be desirable to immobilize either the sphingosine kinase-like protein polypeptide (or polynucleotide) or the test compound to facilitate separation of bound from unbound forms of one or both of the interactants, as well as to accommodate automation of the assay. Thus, either the sphingosine kinase-like protein polypeptide (or polynucleotide) or the test compound can be bound to a solid support. Suitable solid supports include, but are not limited to, glass or plastic slides, tissue culture plates, microtiter wells, tubes, silicon chips, or particles such as beads (including, but not limited to, latex, polystyrene, or glass beads). Any method known in the art can be used to attach the sphingosine kinase-like protein polypeptide (or polynucleotide) or test compound to a solid support, including use of covalent and non-covalent linkages, passive absorption, or pairs of binding moieties attached respectively to the polypeptide or test compound and the solid support. Test compounds are preferably bound to the solid support in an array, so that the location of individual test compounds can be tracked. Binding of a test compound to a sphingosine kinase-like protein polypeptide (or polynucleotide) can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and microcentrifuge tubes.

In one embodiment, a sphingosine kinase-like protein polypeptide is a fusion protein comprising a domain that allows the sphingosine kinase-like protein polypeptide to be bound to a solid support. For example, glutathione-S-transferase fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, Mo.)

or glutathione derivatized microtiter plates, which are then combined with the test compound or the test compound and the non-adsorbed sphingosine kinase-like protein polypeptide; the mixture is then incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components. Binding of the interactants can be determined either directly or indirectly, as described above. Alternatively, the complexes can be dissociated from the solid support before binding is determined.

Other techniques for immobilizing polypeptides or polynucleotides on a solid support also can be used in the screening assays of the invention. For example, either a sphingosine kinase-like protein polypeptide (or polynucleotide) or a test compound can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated sphingosine kinase-like protein polypeptides or test compounds can be prepared from biotin-NHS(N-hydroxysuccinimide) using techniques well known in the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, Ill.) and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies which specifically bind to a sphingosine kinase-like protein polypeptide polynucleotides, or a test compound, but which do not interfere with a desired binding site, such as the active site or a fibronectin domain of the sphingosine kinase-like protein polypeptide, can be derivatized to the wells of the plate. Unbound target or protein can be trapped in the wells by antibody conjugation.

Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies which specifically bind to the sphingosine kinase-like protein polypeptide (or polynucleotides) or test compound, enzyme-linked assays which rely on detecting a sphingosine kinase-like protein activity of the sphingosine kinase-like protein polypeptide, and SDS gel electrophoresis under non-reducing conditions.

Screening for test compounds which bind to a sphingosine kinase-like protein polypeptide or polynucleotide also can be carried out in an intact cell. Any cell which comprises a sphingosine kinase-like protein polynucleotide or polypeptide can be used in a cell-based assay system. A sphingosine kinase-like protein polynucleotide can be naturally occurring in the cell or can be introduced using techniques such as those described above. Either a primary culture or an established cell line, including neoplastic cell lines such as the colon cancer cell lines HCT116, DLD1, HT29, Caco2, SW837, SW480, and RKO, breast cancer cell lines 21-PT, 21-MT, MDA-468, SK-BR3, and BT-474, the A549 lung cancer cell line, and the H392 glioblastoma cell line, can be used. An intact cell is contacted with a test compound. Binding of the test compound to a sphingosine kinase-like protein polypeptide or polynucleotide is determined as described above, after lysing the cell to release the sphingosine kinase-like protein polypeptide-test compound complex.

Enzyme Assays

Test compounds can be tested for the ability to increase or decrease a sphingosine kinase-like protein activity of a sphingosine kinase-like protein polypeptide. Sphingosine kinase-like protein activity can be measured, for example, as described in Liu *et al., J. Biol. Chem. 275*, 19513-20, 2000. Sphingosine kinase-like protein activity can be measured after contacting either a purified sphingosine kinase-like protein polypeptide, a cell extract, or an intact cell with a test compound. A test compound which decreases sphingosine kinase-like protein activity by at least about 10, preferably about 50, more preferably about 75, 90, or 100% is identified as a potential therapeutic agent for decreasing intracellular signaling. A test compound which increases sphingosine kinase-like protein activity by at least about 10, preferably about 50, more preferably about 75, 90, or 100% is identified as a potential therapeutic agent for increasing intracellular signaling.

Gene Expression

In another embodiment, test compounds which increase or decrease sphingosine kinase-like protein gene expression are identified. A sphingosine kinase-like protein polynucleotide is contacted with a test compound, and the expression of an RNA or polypeptide product of the sphingosine kinase-like protein polynucleotide is determined. The level of expression of sphingosine kinase-like protein mRNA or polypeptide in the presence of the test compound is compared to the level of expression of sphingosine kinase-like protein mRNA or polypeptide in the absence of the test compound. The test compound can then be identified as a modulator of expression based on this comparison. For example, when expression of sphingosine kinase-like protein mRNA or polypeptide is greater in the presence of the test compound than in its absence, the test compound is identified as a stimulator or enhancer of sphingosine kinase-like protein mRNA or polypeptide is less expression. Alternatively, when expression of the mRNA or protein is less in the presence of the test compound than in its absence, the test compound is identified as an inhibitor of sphingosine kinase-like protein mRNA or polypeptide expression.

The level of sphingosine kinase-like protein mRNA or polypeptide expression in the cells can be determined by methods well known in the art for detecting mRNA or protein. Either qualitative or quantitative methods can be used. The presence of polypeptide products of a sphingosine kinase-like protein polynucleotide can be determined, for example, using a variety of techniques known in the art, including immunochemical methods such as radioimmunoassay, Western blotting, and immunohistochemistry. Alternatively, polypeptide synthesis can be determined *in vivo*, in a cell culture, or in an *in vitro* translation system by detecting incorporation of labeled amino acids into a sphingosine kinase-like protein polypeptide.

Such screening can be carried out either in a cell-free assay system or in an intact cell. Any cell which expresses a sphingosine kinase-like protein polynucleotide can be used in a cell-based assay system. The sphingosine kinase-like protein poly-

nucleotide can be naturally occurring in the cell or can be introduced using techniques such as those described above. Either a primary culture or an established cell line, including neoplastic cell lines such as the colon cancer cell lines HCT116, DLD1, HT29, Caco2, SW837, SW480, and RKO, breast cancer cell lines 21-PT, 21-MT, MDA-468, SK-BR3, and BT-474, the A549 lung cancer cell line, and the H392 glioblastoma cell line, can be used.

Pharmaceutical Compositions

The invention also provides pharmaceutical compositions which can be administered to a patient to achieve a therapeutic effect. Pharmaceutical compositions of the invention can comprise a sphingosine kinase-like protein polypeptide, sphingosine kinase-like protein polynucleotide, antibodies which specifically bind to a sphingosine kinase-like protein polypeptide, or mimetics, agonists, antagonists, or inhibitors of a sphingosine kinase-like protein polypeptide. The compositions can be administered alone or in combination with at least one other agent, such as stabilizing compound, which can be administered in any sterile, biocompatible pharmaceutical carrier, including, but not limited to, saline, buffered saline, dextrose, and water. The compositions can be administered to a patient alone, or in combination with other agents, drugs or hormones.

In addition to the active ingredients, these pharmaceutical compositions can contain suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Pharmaceutical compositions of the invention can be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, parenteral, topical, sublingual, or rectal means. Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be

formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

Pharmaceutical preparations for oral use can be obtained through combination of active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, or sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethylcellulose; gums including arabic and tragacanth; and proteins such as gelatin and collagen. If desired, disintegrating or solubilizing agents can be added, such as the cross-linked polyvinyl pyrrolidone, agar, alginic acid, or a salt thereof, such as sodium alginate.

Dragee cores can be used in conjunction with suitable coatings, such as concentrated sugar solutions, which also can contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments can be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, *i.e.*, dosage.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with a filler or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds can be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

Pharmaceutical formulations suitable for parenteral administration can be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks'

solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions can contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds can be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Non-lipid polycationic amino polymers also can be used for delivery. Optionally, the suspension also can contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions. For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

The pharmaceutical compositions of the present invention can be manufactured in a manner that is known in the art, *e.g.*, by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes. The pharmaceutical composition can be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, etc. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms. In other cases, the preferred preparation can be a lyophilized powder which can contain any or all of the following: 1-50 mM histidine, 0.1%-2% sucrose, and 2-7% mannitol, at a pH range of 4.5 to 5.5, that is combined with buffer prior to use.

Further details on techniques for formulation and administration can be found in the latest edition of REMINGTON'S PHARMACEUTICAL SCIENCES (Maack Publishing Co., Easton, Pa.). After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated condition. Such labeling would include amount, frequency, and method of administration.

Therapeutic Indications and Methods

The novel human sphingosine kinase-like protein sequence, and in particular the sequence at the 5' end of the mRNA can be used in antisense nucleic acid production, diagnosis of over- or under-expression of human sphingosine kinase-like protein, and in detecting disease-related mutations in the human sphingosine kinase-like protein gene. The sequence can also be transfected into host cells to produce human sphingosine kinase-like protein-expressing cells for use in drug screening. Regulators of human sphingosine kinase-like protein can be used as agents to treat inflammation related to allergy, infection, trauma, or exposure to toxic compounds, or to treat diseases such as infection, hereditary diseases, autoimmune diseases, cancer, neurodegenerative diseases, and cardiovascular diseases. More specifically, human sphingosine kinase-like protein-regulating agents can be beneficial in the prevention of tumor proliferation and invasion and in the promotion of tumor cell apoptosis; in the inhibition of angiogenesis in disease states such as rheumatoid arthritis, diabetic retinopathy, psoriasis, and cancer, and in the promotion of new blood vessel growth in heart disease, wound healing, and bone marrow transplantation; in the regulation of smooth muscle cell proliferation in disease states such as atherosclerosis and asthma; in the modulation of cellular responsiveness to stress such as in ischemic tissue injury; in the maintenance of cardiac function; and in the regulation of vesicular trafficking, tissue invasion, activation, and apoptosis of inflammatory cells.

1. Allergies. Regulation of sphingosine kinase-like protein activity provides a method of treating allergies. The first step in the pathogenesis of an allergic response is the production of immunoglobulin E (IgE) antibody in response to an allergen. Upon exposure to allergens the B cells of responsive individuals secrete IgE molecules specific to the allergen. IgE molecules bind to the high affinity IgE receptor (FcRI) present on mast cells and basophils. (*See* U.S. Patent No. 5,977,072).

IgE binding activates the release of a variety of vasoactive mediators which promote allergic and inflammatory responses. Activation occurs whenever two or more FcRIs are crosslinked via bound IgE molecules that in turn form an aggregate with an allergen molecule. Such aggregation initiates a biochemical cascade which releases histamine and proteases from cytoplasmic granules and leads to the synthesis of prostaglandins, leukotrienes, cytokines and other effectors of the hypersensitivity response.

Mast cells and basophils accumulate at sites of inflammation and, upon activation, secrete hematopoietic growth factors such as granulocyte/-macrophage colony-stimulating factor, interleukin-3, and interleukin-6. These factors propagate the inflammatory response by recruiting, priming, and activating inflammatory cells such as neutrophils, macrophages and eosinophils. The activated cells accumulate in areas of ongoing inflammation, tumor invasion, angiogenesis, fibrosis, and thrombosis. The IgE-dependent activation of cells via FcRI results in an inflammatory response directed towards local tissue defense, tissue maintenance and remodeling, and immunoregulation (Gagari, E. et al (1997) Blood 89:2654-2663).

IgE binding to the FcRI activates kinases which are bound to the receptor under resting conditions. The phosphorylated receptor activates sphingosine kinase, which induces the production of sphingosine-1-phosphate (SPP) and contributes to calcium mobilization in mast cells. The phosphorylated receptor also activates tyrosine kinases, such as Lyn and Syk, which induce the tyrosine phosphorylation of cytoplasmic molecules including phospholipase C. Phosphorylated phospholipase C hydrolyses phosphatidylinositol 4,5-bisphosphate and liberates inositol 1,4,5-trisphosphate and diacylglycerol. The latter mobilizes Ca^{2+} from intracellular and extracellular sources and activates protein kinase C (Paolini, R. et al. (1991) Nature 353: 855-858; and Beaven, M. A. and Baumgartner, R. A. (1996) Curr. Opin. Immunol. 8:766-772).

SPP has diverse biological functions, acting both inside cells as a second messenger to regulate Ca^{2+} mobilization, cell activation, proliferation, and survival, and extracellularly as a ligand for GPCRs of the EDG family (EDG-1,3,5,6 and 8). SPP is synthesized in response to extracellular stimuli by the sequential action of sphingomyelinase, ceramidase, and sphingosine kinase. Many lines of evidence have shown that sphingosine kinase activity is regulated at both the transcription and translation levels and plays crucial roles in S1P production.

2. The sphingosine kinases may be important in the pathogenesis of asthma and other inflammatory diseases. Significant elevations in SPP levels in bronchoalveolar lavage fluid have been observed in asthmatic subjects following segmental antigen challenge. SPP (itself or in synergy with PDGF) has also been reported to regulate airway smooth muscle proliferation, contraction, and IL-6 production. Additionally, it has been demonstrated that the balance between sphingosine and S1P is decisive for the activation of mast cells, a cell type implicated as an initiator of many asthmatic symptoms, where Sphingosine kinase1 acts as a permissive switch for their stimulation. Inhibition of sphingosine kinases in mast cells leads to a decrease in Ca^{2+} mobilization, mast cell degranulation, and leukotriene and cytokine synthesis and secretion. Similarly, inhibition of sphingosine kinase activity in endothelial cells leads to the decreased expression of the adhesion molecules E-selectin and VCAM-1 induced by TNF-α.

Sphingosine kinase-like protein resembles the two known sphingosine kinases in possessing a diacylglycerol kinase catalytic domain which is presumed to give sphingosine kinases the ability to phosphorylate D-threo-sphingosine. It differs from the other two sphingosine kinases, however, in additionally having a pleckstrin homology (PH) domain at its N-terminal (see diagram below). PH is a domain commonly found in signaling proteins and

has multiple functions including the ability to bind inositol phosphates, beta/gamma subunits of G proteins, phosphorylated Ser/Thr residues of proteins, and membranes. The three human sphingosine kinases, SPHK1, SPHK 2, and sphingosine kinase-like, encode proteins of 384, 618, and 537 amino acids respectively. In addition to the differences in length, the sphingosine kinases also show differences in tissue distribution. Of the three, sphingosine kinase-like appears to have the broadest distribution, being found expressed in nearly all tissues tested. Notably, however, it is expressed highest in microvascular endothelial cells, a cell type found throughout the body. This indicates that sphingosine kinase-like, more so than other sphingosine kinases, may play an important role in the activation of endothelial cells, regulating their adhesion molecule expression, cell-to-cell contact, cytokine and growth factor secretion, proliferation, and angiogenesis. Accordingly, sphingosine kinase-like may be a crucial regulator of immune cell trafficking, controlling the interaction between blood leukocytes and endothelial cells, and allowing leukocytes to extravasate into tissues. Regulation of Sphingosine kinase-like would therefore be expected to be beneficial in the treatment of diseases in which there is an overactive or unwanted immune response, or in diseases in which overproduction of cytokines, such as tumor necrosis factor, compromises the intergrity of the vascular endothelium.

3. Cancer. Regulation of sphingosine kinase-like protein activity may provide a method of treating cancer. Cancer is a disease fundamentally caused by oncogenic cellular transformation. There are several hallmarks of transformed cells that distinguish them from their normal counterparts and underlie the pathophysiology of cancer. These include uncontrolled cellular proliferation, unresponsiveness to normal death-inducing signals (immortalization), increased cellular motility and invasiveness, increased ability to recruit blood supply through induction of new blood vessel formation (angiogenesis), genetic instability, and dysregulated gene expression.

Various combinations of these aberrant physiologies, along with the acquisition of drug-resistance frequently lead to an intractable disease state in which organ failure and patient death ultimately ensue.

Most standard cancer therapies target cellular proliferation and rely on the differential proliferative capacities between transformed and normal cells for their efficacy. This approach is hindered by the facts that several important normal cell types are also highly proliferative and that cancer cells frequently become resistant to these agents. Thus, the therapeutic indices for traditional anti-cancer therapies rarely exceed 2.0.

The advent of genomics-driven molecular target identification has opened up the possibility of identifying new cancer-specific targets for therapeutic intervention that will provide safer, more effective treatments for cancer patients. Thus, newly discovered tumor-associated genes and their products can be tested for their role(s) in disease and used as tools to discover and develop innovative therapies. Genes playing important roles in any of the physiological processes outlined above can be characterized as cancer targets.

Genes or gene fragments identified through genomics can readily be expressed in one or more heterologous expression systems to produce functional recombinant proteins. These proteins are characterized *in vitro* for their biochemical properties and then used as tools in high-throughput molecular screening programs to identify chemical modulators of their biochemical activities. Agonists and/or antagonists of target protein activity can be identified in this manner and subsequently tested in cellular and *in vivo* disease models for anti-cancer activity. Optimization of lead compounds with iterative testing in biological models and detailed pharmacokinetic and toxicological analyses form the basis for drug development and subsequent testing in humans.

Increased sphingosine kinase activity is been associated with enhanced cellular proliferation. Thus, downregulation of sphingosine kinase-like protein activity is an attractive therapeutic approach to the treatment and prevention of cancer.

4. Autoimmune disease. The principal physiologic function of the immune system is the elimination of infectious organisms. The effector mechanisms that are responsible for protective immunity are also capable of injuring host tissues. In some situations, specific immune responses have little or no protective value, and the harmful consequences become dominant. The best example of this is autoimmune disease caused by pathologic immune responses against self-antigens. (*See* U.S. Patent No. 6,098,631).

Potentially harmful immune reactions may be prevented either by functionally inactivating or killing the responding lymphocytes. The primary cytolytic mechanism involved in controlling lymphocyte responses is the Fas-mediated apoptotic pathway. Using this pathway, the immune system actively eliminates potentially harmful cells so that the host may survive. See A. Abbas, "Die and Let Live: Eliminating Dangerous Lymphocytes," Cell 84:655 (1996). Abnormalities in Fas-mediated cell death pathways may result in autoimmunity even in situations in which Fas and Fas Ligand are themselves normal. For example, where apoptosis is inhibited and a proliferation pathway is stimulated, activated lymphocytes may escape elimination and cause disease.

Established treatments of autoimmune disease are designed to inhibit either final common pathways of inflammation or immunological mediators. Both approaches are non-specific and, therefore, are associated with severe side effects, such as musculoskeletal, metabolic, neurologic and connective tissue side effects, immuno-suppression, bone marrow and gastrointestinal toxicity, liver damage, lung disease, hypersensitivity reactions, deafness, renal toxicity, mucocutaneous toxicity. *See*, R. Million et al., *Lancet* 1:812 (1984), J. A. Engelbrecht et al., *Arthritis and*

Rheumatism 26:1275 (1983), G. W. Cannon et al., *Arthritis and Rheumatism* 26:1269 (1983), Simon and Mills, "Nonsteroidal Antiinflammatory Drugs," N. Eng. J. Med. 302:1179 (1980), Katz et al., *Ann. Int. Med.* 101:176 (1984), W. F. Kean et al., *Arthritis and Rheumatism* 23:158 (1980).

Thus, current therapies for autoimmune diseases are associated with high incidence of serious side effects. Furthermore, although some medications may offer symptomatic relief, in many cases, they do not significantly modify the progression of symptoms such as joint destruction. What is needed is an effective therapeutic approach with lower toxicity such that the treatment is better tolerated and more appropriate for the treatment of autoimmune diseases.

SPP has been recently shown to inhibit the Fas-mediated cell death pathway. *See* O. Cuvillier et al., "Suppression of ceramide-mediated Programmed Cell Death By Sphingosine-1-phosphate," Nature 381:800 (1996). Thus, inhibition of sphingosine kinase- like protein activity is a viable approach to reversing the inhibition of Fas-mediated apoptosis by preventing the formation of SPP. SPP is produced from sphingosine by the activity of sphingosine kinase. The net effect of SPP is inhibition of ceramide-mediated cell death. Thus, regulation of sphingosine kinase-like protein may provide a method of treating or preventing autoimmune disease such as, for example, rheumatoid arthritis, systemic lupus erythematosus, Grave's disease, multiple sclerosis (MS), and Type I diabetes..

The invention further pertains to the use of novel agents identified by the screening assays described above. Accordingly, it is within the scope of this invention to use a test compound identified as described herein in an appropriate animal model. For example, an agent identified as described herein (e.g., a modulating agent, an antisense nucleic acid molecule, a specific antibody, ribozyme, or a polypeptide-binding partner) can be used in an animal model to determine the efficacy, toxicity, or side effects of treatment with such an agent. Alternatively, an agent identified as described herein can be used in an animal model to determine the mechanism of

action of such an agent. Furthermore, this invention pertains to uses of novel agents identified by the above-described screening assays for treatments as described herein.

A reagent which affects sphingosine kinase-like protein activity can be administered to a human cell, either *in vitro* or *in vivo*, to reduce sphingosine kinase-like protein activity. The reagent preferably binds to an expression product of a human sphingosine kinase-like protein gene. If the expression product is a polypeptide, the reagent is preferably an antibody. For treatment of human cells *ex vivo*, an antibody can be added to a preparation of stem cells which have been removed from the body. The cells can then be replaced in the same or another human body, with or without clonal propagation, as is known in the art.

In one embodiment, the reagent is delivered using a liposome. Preferably, the liposome is stable in the animal into which it has been administered for at least about 30 minutes, more preferably for at least about 1 hour, and even more preferably for at least about 24 hours. A liposome comprises a lipid composition that is capable of targeting a reagent, particularly a polynucleotide, to a particular site in an animal, such as a human. Preferably, the lipid composition of the liposome is capable of targeting to a specific organ of an animal, such as the lung or liver.

A liposome useful in the present invention comprises a lipid composition that is capable of fusing with the plasma membrane of the targeted cell to deliver its contents to the cell. Preferably, the transfection efficiency of a liposome is about 0.5 μg of DNA per 16 nmole of liposome delivered to about 10^6 cells, more preferably about 1.0 μg of DNA per 16 nmol of liposome delivered to about 10^6 cells, and even more preferably about 2.0 μg of DNA per 16 nmol of liposome delivered to about 10^6 cells. Preferably, a liposome is between about 100 and 500 nm, more preferably between about 150 and 450 nm, and even more preferably between about 200 and 400 nm in diameter.

Suitable liposomes for use in the present invention include those liposomes standardly used in, for example, gene delivery methods known to those of skill in the art. More preferred liposomes include liposomes having a polycationic lipid composition and/or liposomes having a cholesterol backbone conjugated to polyethylene glycol. Optionally, a liposome comprises a compound capable of targeting the liposome to a tumor cell, such as a tumor cell ligand exposed on the outer surface of the liposome.

Complexing a liposome with a reagent such as an antisense oligonucleotide or ribozyme can be achieved using methods which are standard in the art (see, for example, U.S. Patent 5,705,151). Preferably, from about 0.1 μg to about 10 μg of polynucleotide is combined with about 8 nmol of liposomes, more preferably from about 0.5 μg to about 5 μg of polynucleotides are combined with about 8 nmol liposomes, and even more preferably about 1.0 μg of polynucleotides is combined with about 8 nmol liposomes.

In another embodiment, antibodies can be delivered to specific tissues *in vivo* using receptor-mediated targeted delivery. Receptor-mediated DNA delivery techniques are taught in, for example, Findeis *et al. Trends in Biotechnol. 11*, 202-05 (1993); Chiou *et al.*, GENE THERAPEUTICS: METHODS AND APPLICATIONS OF DIRECT GENE TRANSFER (J.A. Wolff, ed.) (1994); Wu & Wu, *J. Biol. Chem. 263*, 621-24 (1988); Wu *et al.*, *J. Biol. Chem. 269*, 542-46 (1994); Zenke *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 87*, 3655-59 (1990); Wu *et al.*, *J. Biol. Chem. 266*, 338-42 (1991).

If the reagent is a single-chain antibody, polynucleotides encoding the antibody can be constructed and introduced into a cell either *ex vivo* or *in vivo* using well-established techniques including, but not limited to, transferrin-polycation-mediated DNA transfer, transfection with naked or encapsulated nucleic acids, liposome-mediated cellular fusion, intracellular transportation of DNA-coated latex beads, protoplast fusion, viral infection, electroporation, "gene gun," and DEAE- or calcium phosphate-mediated transfection.

Determination of a Therapeutically Effective Dose

The determination of a therapeutically effective dose is well within the capability of those skilled in the art. A therapeutically effective dose refers to that amount of active ingredient which increases or decreases intracellular signaling relative to that which occurs in the absence of the therapeutically effective dose.

For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays or in animal models, usually mice, rabbits, dogs, or pigs. The animal model also can be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

Therapeutic efficacy and toxicity, *e.g.*, ED_{50} (the dose therapeutically effective in 50% of the population) and LD_{50} (the dose lethal to 50% of the population), can be determined by standard pharmaceutical procedures in cell cultures or experimental animals. The dose ratio of toxic to therapeutic effects is the therapeutic index, and it can be expressed as the ratio, LD_{50}/ED_{50}.

Pharmaceutical compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies is used in formulating a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to the subject that requires treatment. Dosage and administration are adjusted to provide sufficient levels of the active ingredient or to maintain the desired effect. Factors which can be taken into account include the severity of the disease state,

general health of the subject, age, weight, and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Long-acting pharmaceutical compositions can be administered every 3 to 4 days, every week, or once every two weeks depending on the half-life and clearance rate of the particular formulation.

Normal dosage amounts can vary from 0.1 to 100,000 micrograms, up to a total dose of about 1 g, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

Effective *in vivo* dosages of an antibody are in the range of about 5 µg to about 50 µg/kg, about 50 µg to about 5 mg/kg, about 100 µg to about 500 µg/kg of patient body weight, and about 200 to about 250 µg/kg of patient body weight. For administration of polynucleotides encoding single-chain antibodies, effective *in vivo* dosages are in the range of about 100 ng to about 200 ng, 500 ng to about 50 mg, about 1 µg to about 2 mg, about 5 µg to about 500 µg, and about 20 µg to about 100 µg of DNA.

If the expression product is mRNA, the reagent is preferably an antisense oligonucleotide or a ribozyme. Polynucleotides which express antisense oligonucleotides or ribozymes can be introduced into cells by a variety of methods, as described above.

Preferably, a reagent reduces expression of a sphingosine kinase-like protein polynucleotide or activity of a sphingosine kinase-like protein polypeptide by at least about 10, preferably about 50, more preferably about 75, 90, or 100% relative to the absence of the reagent. The effectiveness of the mechanism chosen to decrease the

level of expression of a sphingosine kinase-like protein polynucleotide or the activity of a sphingosine kinase-like protein polypeptide can be assessed using methods well known in the art, such as hybridization of nucleotide probes to sphingosine kinase-like protein-specific mRNA, quantitative RT-PCR, immunologic detection of a sphingosine kinase-like protein polypeptide, or measurement of sphingosine kinase-like protein activity.

In any of the embodiments described above, any of the pharmaceutical compositions of the invention can be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy can be made by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents can act synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

Any of the therapeutic methods described above can be applied to any subject in need of such therapy, including, for example, mammals such as dogs, cats, cows, horses, rabbits, monkeys, and most preferably, humans.

The above disclosure generally describes the present invention, and all patents and patent applications cited in this disclosure are expressly incorporated herein. A more complete understanding can be obtained by reference to the following specific examples which are provided for purposes of illustration only and are not intended to limit the scope of the invention.

EXAMPLE 1

Detection of sphingosine kinase-like protein activity

The polynucleotide of SEQ ID NO: 1 is inserted into the expression vector pCEV4 and the expression vector pCEV4-sphingosine kinase-like protein polypeptide obtained is transfected into human embryonic kidney 293 cells. From these cells extracts are obtained and sphingosine kinase-like protein activity is determined for 20 min at 37°C in a total volume of 200 µl, containing 10 µM [3H]sphingosine (~105 cpm), 1 mM ATP, 10 mM MgCl2, 1 mM EDTA, 1 mM 2-mercaptoethanol, 1 mg/ml fatty acid-free bovine serum albumin, 100mM patassium phosphate buffer (pH 7.4), and HL-60 cytosol (120-200 µg of protein). Reactions are terminated by addition of 2 ml of ice-cold methanol. Extraction, TLC separation, and quantification of [3H]SPP are carried out. Sphingosine kinase-like protein activity is expressed as pmol SPP formed per mg protein and 20 min. protein. It is shown that the polypeptide of SEQ ID NO: 2 has a sphingosine kinase-like protein activity.

EXAMPLE 2

Identification of a test compound which binds to a sphingosine kinase-like protein polypeptide

Purified sphingosine kinase-like protein polypeptides comprising a glutathione-S-transferase protein are absorbed onto glutathione-derivatized wells of 96-well microtiter plates are contacted with test compounds from a small molecule library at pH 7.0 in a physiological buffer solution. Sphingosine kinase-like protein polypeptides comprise the amino acid sequence shown in SEQ ID NOS: 2, 10, and 11. The test compounds comprise a fluorescent tag. The samples are incubated for 5 minutes to one hour. Control samples are incubated in the absence of a test compound.

The buffer solution containing the test compounds is washed from the wells. Binding of a test compound to a sphingosine kinase-like protein polypeptide is detected by fluorescence measurements of the contents of the wells. A test compound which increases the fluorescence in a well by at least 15% relative to fluorescence of a well in which a test compound was not incubated is identified as a compound which binds to a sphingosine kinase-like protein polypeptide.

EXAMPLE 3

Identification of a test compound which decreases sphingosine kinase-like protein activity

HEK293 cells are seeded at 6 x 10^5/well in poly-L-lysine-coated 6-well plates and cultured for 24 hours. Cells are then transfected with vectors containing sphingosine kinase-like constructs and 6 µl LipofectAMINE Plus reagent. An additional 4 µl LipofectAMINE Plus reagent is added to each well as well as a suitable amount of a test compound from a small molecule library. Control wells receive no test compound. 1-3 days post-transfection, cells are harvested and lysed by freeze-thawing. Sphingosine kinase-like protein activity is measured in the presence of sphingosine. A test compound which decreases sphingosine kinase-like protein activity of the extract relative to the control extract by at least 20% is identified as a sphingosine kinase-like protein inhibitor. *See* Liu *et al., J. Biol. Chem. 275*, 19513-20, 2000.

EXAMPLE 4

Identification of a test compound which decreases sphingosine kinase-like protein gene expression

A test compound is administered to a primary culture of MC3T3-El osteoblast cells and incubated at 37°C for 10 to 45 minutes. A culture of the same type of cells incubated for the same time without the test compound provides a negative control.

RNA is isolated from the two cultures as described in Chirgwin *et al.*, *Biochem. 18*, 5294-99, 1979). Northern blots are prepared using 20 to 30 µg total RNA and hybridized with a ^{32}P-labeled sphingosine kinase-like protein-specific probe at 65°C in Express-hyb (CLONTECH). The probe comprises at least 11 contiguous nucleotides selected from the complement of SEQ ID NOS: 1 and 9. A test compound which decreases the sphingosine kinase-like protein-specific signal relative to the signal obtained in the absence of the test compound is identified as an inhibitor of sphingosine kinase-like protein gene expression.

EXAMPLE 5

Treatment of a tumor with a reagent which specifically binds to a sphingosine kinase-like protein gene product

Synthesis of antisense sphingosine kinase-like protein oligonucleotides comprising at least 11 contiguous nucleotides selected from the complement of SEQ ID NOS: 1 and 9 is performed on a Pharmacia Gene Assembler series synthesizer using the phosphoroamidite procedure (Uhlmann *et al.*, *Chem. Rev. 90*, 534-83, 1990). Following assembly and deprotection, oligonucleotides are ethanol-precipitated twice, dried, and suspended in phosphate-buffered saline (PBS) at the desired concentration. Purity of these oligonucleotides is tested by capillary gel electrophoreses and ion exchange HPLC. Endotoxin levels in the oligonucleotide preparation are determined using the *Limulus* Amebocyte Assay (Bang, *Biol. Bull. (Woods Hole, Mass.) 105*, 361-362, 1953).

A composition containing the antisense oligonucleotides at a concentration of 0.1-100 µM is administered directly into the tumor. Tumor size is monitored over a

period of days or weeks. Additional doses of the antisense oligonucleotides can be given during that time. Tumor growth is suppressed due to decreased sphingosine kinase-like protein activity.

EXAMPLE 6

Treatment of a rheumatoid arthritis with a reagent which specifically binds to a sphingosine kinase-like protein gene product

Synthesis of antisense sphingosine kinase-like protein oligonucleotides comprising at least 11 contiguous nucleotides selected from the complement of SEQ ID NOS: 1 and 9 is performed on a Pharmacia Gene Assembler series synthesizer using the phosphoroamidite procedure (Uhlmann *et al.*, *Chem. Rev. 90*, 534-83, 1990). Following assembly and deprotection, oligonucleotides are ethanol-precipitated twice, dried, and suspended in phosphate-buffered saline (PBS) at the desired concentration. Purity of these oligonucleotides is tested by capillary gel electrophoreses and ion exchange HPLC. Endotoxin levels in the oligonucleotide preparation are determined using the *Limulus* Amebocyte Assay (Bang, *Biol. Bull. (Woods Hole, Mass.) 105*, 361-362, 1953).

An aqueous composition containing the antisense oligonucleotides at a concentration of 0.1-100 μM is administered to the patient using a needle.

Severity of rheumatoid arthritis atherosclerosis is monitored over a period of days or weeks by removing synovial fluid from the knee joint, isolating synovial T cells, and determining whether the T cells are resistant to Fas-mediated DNA fragmentation. Briefly, the T cells were lysed in TE buffer containing 0.2% Triton X -100, pH 8. Fragmented DNA was separated from intact chromatin by microfuging for 20 min, 14,000 rpm at 4°C. The resulting supernatant is treated with 1 mg/ml of proteinase K at 37°C overnight, then extracted with phenol/chloroform/isoamyl alcohol (25:24:1) three times. DNA is precipitated by addition of three volumes of absolute ethanol, in

the presence of 0.3 M sodium acetate, pH 5.2, incubated overnight at -20°C and then pelleted by centrifugation at 14,000 rpm at 4°C for 20 min. The pellet is washed twice with 75% ethanol and dissolved in 30 µl of TE containing 10 µg/ml of RNase overnight at 37°C. DNA samples are separated by electrophoresis on 1.8% agarose gel in the presence of ethidium bromide. Additional injections of the antisense oligonucleotides can be given during that time. Rheumatoid arthritis is suppressed due to decreased sphingosine kinase-like protein activity.

EXAMPLE 7

Proliferation inhibition assay: Antisense oligonucleotides suppress the growth of cancer cell lines

The cell line used for testing is the human colon cancer cell line HCT116. Cells are cultured in RPMI-1640 with 10-15% fetal calf serum at a concentration of 10,000 cells per milliliter in a volume of 0.5 ml and kept at 37 °C in a 95% air/5%CO_2 atmosphere.

Phosphorothioate oligoribonucleotides are synthesized on an Applied Biosystems Model 380B DNA synthesizer using phosphoroamidite chemistry. A sequence of 24 bases is used as the test oligonucleotide: (1) 5'-TGG TTT CGT AAA TGA CCA TAA ATA-3' (complementary to the nucleotides at position 1 to 24 of SEQ ID NOS: 2, 10, and 11). As a control, another (random) sequence is used: 5'-TCA ACT GAC TAG ATG TAC ATG GAC-3'. Following assembly and deprotection, oligonucleotides are ethanol-precipitated twice, dried, and suspended in phosphate buffered saline at the desired concentration. Purity of the oligonucleotides is tested by capillary gel electrophoresis and ion exchange HPLC. The purified oligonucleotides are added to the culture medium at a concentration of 10 µM once per day for seven days.

The addition of the test oligonucleotide for seven days results in significantly reduced expression of human sphingosine kinase-like protein as determined by Western blotting. This effect is not observed with the control oligonucleotide. After 3 to 7 days, the number of cells in the cultures is counted using an automatic cell counter. The number of cells in cultures treated with the test oligonucleotide (expressed as 100%) is compared with the number of cells in cultures treated with the control oligonucleotide. The number of cells in cultures treated with the test oligonucleotide is not more than 30% of control, indicating that the inhibition of human sphingosine kinase-like protein has an anti-proliferative effect on cancer cells.

EXAMPLE 8

Quantitative Expression Profiling

Expression profiling is based on a quantitative polymerase chain reaction (PCR) analysis, also called kinetic analysis, first described in Higuchi et al., 1992 and Higuchi et al., 1993. The principle is that at any given cycle within the exponential phase of PCR, the amount of product is proportional to the initial number of template copies. Using this technique, the expression levels of particular genes, which are transcribed from the chromosomes as messenger RNA (mRNA), are measured by first making a DNA copy (cDNA) of the mRNA, and then performing quantitative PCR on the cDNA, a method called quantitative reverse transcription-polymerase chain reaction (quantitative RT-PCR).

Quantitative RT-PCR analysis of RNA from different human tissues was performed to investigate the tissue distribution of LBRI-221 (SEQ ID NOS: 2, 10, and 11), Sphingosine kinase-like mRNA. In most cases, 25 μg of total RNA from various tissues (including Human Total RNA Panel I-V, Clontech Laboratories, Palo Alto, CA, USA) was used as a template to synthesize first-strand cDNA using the SUPERSCRIPT™ First-Strand Synthesis System for RT-PCR (Life Technologies, Rockville , MD, USA). First-strand cDNA synthesis was carried out according to the

manufacturer's protocol using oligo (dT) to hybridize to the 3' poly A tails of mRNA and prime the synthesis reaction. Approximately 10 ng of the first-strand cDNA was then used as template in a polymerase chain reaction. In other cases, 10 ng of commercially available cDNAs (Human Immune System MTC Panel and Human Blood Fractions MTC Panel, Clontech Laboratories, Palo Alto, CA, USA) were used as template in a polymerase chain reaction. The polymerase chain reaction was performed in a LightCycler (Roche Molecular Biochemicals, Indianapolis, IN, USA), in the presence of the DNA-binding fluorescent dye SYBR Green I which binds to the minor groove of the DNA double helix, produced only when double-stranded DNA is successfully synthesized in the reaction (Morrison et al., 1998). Upon binding to double-stranded DNA, SYBR Green I emits light that can be quantitatively measured by the LightCycler machine. The polymerase chain reaction was carried out using oligonucleotide primers LBRI221-L3 (SEQ ID NO: 12) and LBRI221-R2 (SEQ ID NO: 13) and measurements of the intensity of emitted light were taken following each cycle of the reaction when the reaction had reached a temperature of 85°C. Intensities of emitted light were converted into copy numbers of the gene transcript per nanogram of template cDNA by comparison with simultaneously reacted standards of known concentration.

To correct for differences in mRNA transcription levels per cell in the various tissue types, a normalization procedure was performed using similarly calculated expression levels in the various tissues of five different housekeeping genes: glyceraldehyde-3-phosphatase (G3PDH), hypoxanthine guanine phophoribosyl transferase (HPRT), beta-actin, porphobilinogen deaminase (PBGD), and beta-2-microglobulin. The level of housekeeping gene expression is considered to be relatively constant for all tissues (Adams et al., 1993, Adams et al., 1995, Liew et al., 1994) and therefore can be used as a gauge to approximate relative numbers of cells per .mug of total RNA used in the cDNA synthesis step. Except for the use of a slightly different set of housekeeping genes and the use of the LightCycler system to measure expression levels, the normalization procedure was similar to that described in the RNA Master Blot User Manual, Appendix C (1997, Clontech Laboratories,

Palo Alto, CA, USA). In brief, expression levels of the five housekeeping genes in all tissue samples were measured in three independent reactions per gene using the LightCycler and a constant amount (25 µg) of starting RNA. The calculated copy numbers for each gene, derived from comparison with simultaneously reacted standards of known concentrations, were recorded and the mean number of copies of each gene in all tissue samples was determined. Then for each tissue sample, the expression of each housekeeping gene relative to the mean was calculated, and the average of these values over the five housekeeping genes was found. A normalization factor for each tissue was then calculated by dividing the final value for one of the tissues arbitrarily selected as a standard by the corresponding value for each of the tissues. To normalize an experimentally obtained value for the expression of a particular gene in a tissue sample, the obtained value was multiplied by the normalization factor for the tissue tested. This normalization method was used for all tissues except those derived from the Human Blood Fractions MTC Panel, which showed dramatic variation in some housekeeping genes depending on whether the tissue had been activated or not. In these tissues, normalization was carried out with a single housekeeping gene, beta-2-microglobulin.

Results are given in Figs. 14 and 15, showing the experimentally obtained copy numbers of mRNA per 10 ng of first-strand cDNA on the left and the normalized values on the right. RNAs used for the cDNA synthesis, along with their supplier and catalog numbers are shown in tables 1 and 2.

EXAMPLE 9

Treatment of asthma with a reagent that specifically binds to a sphingosine kinase-like protein gene product

Synthesis of antisense sphingosine kinase-like protein oligonucleotides comprising at least 11 contiguous nucleotides selected from the complement of SEQ ID NOS: 1 and 9 is performed on a Pharmacia Gene Assembler series synthesizer using the

phosphoroamidite procedure (Uhlmann *et al.*, *Chem. Rev. 90*, 534-83, 1990). Following assembly and deprotection, oligonucleotides are ethanol-precipitated twice, dried, and suspended in phosphate-buffered saline (PBS) at the desired concentration. Purity of these oligonucleotides is tested by capillary gel electrophoreses and ion exchange HPLC. Endotoxin levels in the oligonucleotide preparation are determined using the *Limulus* Amebocyte Assay (Bang, *Biol. Bull. (Woods Hole, Mass.) 105*, 361-362, 1953).

An aqueous composition containing the antisense oligonucleotides is administered to the patient by inhalation.

Severity of asthma is monitored over a period of days or weeks by noting changes in patients' asthmatic symptoms, measuring lung function, or measuring changes in markers of lung inflammation such as numbers of inflammatory cells or concentrations of inflammatory mediators in fluid sampled from patients' lungs by bronchoalveolar lavage. Asthma severity is reduced due to decreased sphingosine kinase-like protein activity.

EXAMPLE 10

In vivo validation of novel compounds

1. Tests for activity of T cells are used to evaluate agents that modulate the expression or activity of costimulatory molecules-cytokines, cytokine receptors, signalling molecules, or other molecules involved in T cell activation.

Mouse anti-CD3-induced cytokine production model:

BALB/c mice are injected with a single intravenous injection of 10 µg of 145-2C11 (purified hamster anti-mouse CD3 monoclonal antibodies,

PHARMINGEN). Compound is administered intraperitoneally 60 min prior to the anti-CD3 mAb injection. Blood is collected 90 min after the antibody injection. Serum is obtained by centrifugation at 3000 r.p.m. for 10 min. Serum levels of cytokines, such as IL-2 and IL-4, or other secreted molecules are determined by an ELISA. Proteins which regulate the CD3 downstream signaling can be evaluated in this model.

2. Tests for activity of B cells are used to evaluate agents that modulate the expression or activity of the B cell receptor, signaling molecules, or other molecules involved in B cell activation/immunoglobulin class switching.

Mouse anti-IgD induced IgE production model:

BALB/c mice are injected intravenously with 0.8 mg of purified goat anti-mouse IgD antibody or PBS (defined as day 0). Compound is administered intraperitoneally from day 0 to day 6. On day 7 blood is collected and serum is obtained by centrifugation at 3000 r.p.m. for 10 min. Serum levels of total IgE are determined by YAMASA's ELISA kit and other Ig subtypes are measured by an Ig ELISA KIT (Rougier Bio-tech's, Montreal, Canada). Proteins that regulate IgD downstream signaling and Ig class switching can be evaluated.

3. Tests for activity of monocytes/macrophages are used to evaluate agents that modulate the expression or activity of signalling molecules, transcription factors.

Mouse LPS-induced TNF-α production model:

Compound is administered to BALB/c mice by intraperitoneal injection and one hour later the mice given LPS (200 μg/mouse) by intraperitoneal injection. Blood is collected 90 minutes after the LPS injection and plasma is

obtained. TNF-α concentration in the sample is determined using an ELISA kit. Proteins that regulate downstream effects of LPS stimulation, such as NF-κB activation, can be evaluated.

4. Tests for activity of eosinophils are used to evaluate agents that modulate the expression or activity of the eotaxin receptor, signaling molecules, cytoskeletal molecules, or adhesion molecules.

Mouse eotaxin-induced eosinophilia model:

BALB/c mice are injected intradermally with a 2.5 ml of air on days –6 and –3 to prepare an airpouch. On day 0, compound is administered intraperitoneally, and 30 minutes later, IL-5 (300 ng/mouse) is injected intravenously. After an additional 30 minutes, eotaxin is injected (3 μg/mouse, i.d.). Four hours after the eotaxin injection, leukocytes in the airpouch exudate are collected and the number of total cells is counted. Differential cell counts in the exudate are performed by staining with May-Grunwald Gimsa solution. Proteins that regulate signaling by the eotaxin receptor or regulate eosinophil trafficking can be evaluated.

5. Passive cutaneous anaphylaxis (PCA) test in rats

6 Weeks old male Wistar rats are sensitized intradermally (i.d.) on their shaved backs with 50 μl of 0.1 μg/ml mouse anti-DNP IgE monoclonal antibody (SPE-7) under a light anesthesia. After 24 hours, the rats are challenged intravenously with 1 ml of saline containing 0.6 mg DNP-BSA (30) (LSL CO., LTD) and 0.005 g of Evans blue. Compounds are injected intraperitoneally (i.p.) 0.5 hr prior to antigen injection. Rats without the sensitization, challenge, and compound treatment are used as a control and rats with sensitization, challenge and vehicle treatment are used to determine the value without inhibition. Thirty minutes after the challenge, the rats are sacrificed, and the skin of the back is removed. Evans blue dye in the skin is

extracted in formamide overnight at 63°C. Absorbance at 620 nm is then measured to obtain the optical density of the leaked dye.

Percent inhibition of PCA with a compound is calculated as follows:

% inhibition = {(mean vehicle value – sample value)/(mean vehicle value – mean control value)} x 100

Proteins that regulate mast cell degranulation, vascular permeability, or receptor antagonists against histamine receptors, serotonin receptors, or cysteinyl leukotriene receptors can be evaluated.

6. Anaphylactic bronchoconstriction in rats

6 Weeks old male Wistar rats are sensitized intravenously (i.v.) with 10 μg mouse anti-DNP IgE, SPE-7, and 1 days later, the rats are challenged intravenously with 0.3 ml of saline containing 1.5 mg DNP-BSA (30) under anesthesia with urethane (1000 mg/kg, i.p.) and gallamine (50 mg/kg, i.v.). The trachea is cannulated for artifical respiration (2 ml/stroke, 70 strokes/min). Pulmonary inflation pressure (PIP) is recorded thruogh a side-arm of the cannula connected to a pressure transducer. Changes in PIP reflect a change of both resistance and compliance of the lungs. To evaluate a compound, the compound is given i.v. 5 min before challenge.

Proteins that regulate mast cell degranulation, vascular permeability or receptor antagonists against histamine receptors, serotonin receptors, or cysteinyl leukotriene receptors can be evaluated. Proteins that regulate the contraction of smooth muscle can be also evaluated.

7. *T cell adhesion to smooth muscle cells or endothelial cells*

A purified population of T cells is prepared by ficoll density centrifugation followed by separation on a nylon wool column, rosetting with sheep red blood cells, or using magnetic beads coated with antibodies. The T cells are activated with mitogen for 36 to 42 hours and labeled with ^{3}H-thymidine during the last 16 hours of the activation. Airway smooth muscle cells or bronchial microvascular endothelial cells are obtained from lung transplant tissue, from bronchus resections from cancer patients, from cadavers, or as cell lines from commercial sources. If fresh tissue is used as the source of cells, the smooth muscle cells and endothelial cells can be isolated from tissue by dissection followed by digestion for 30-60 minutes in a solution containing 1.7 mM ethyleneglycol-bis-(beta-aminoethylether)-N,N,N',N'-tetraacetic acid, 640 U/ml collagenase, 10 mg/ml soybean trypsin inhibitor, and 10 U/ml elastase. The smooth muscle cells or endothelial cells are grown in 24-well tissue culture dishes until confluent and then treated with a test compound and inflammatory mediators, such as TNF-α, for 24 hours. To measure adhesion, 6×10^{5} T cells are added per well and allowed to adhere for one hour at 37°C. Nonadherent cells are removed by washing six times gently with medium. Finally, the remaining adherent cells are lysed by adding 300 µl 1% Triton-X 100 in PBS to each well and quantitating the radioactivity in a scintillation counter. The percent binding is calculated as counts recovered from adherent cells/total input counts x 100%.

Table 1: **Whole-body-screen tissues**

Tissue	Supplier	Panel name and catalog number
1. brain	Clontech	Human Total RNA Panel I, K4000-1
2. heart	Clontech	Human Total RNA Panel I, K4000-1
3. kidney	Clontech	Human Total RNA Panel I, K4000-1
4. liver	Clontech	Human Total RNA Panel I, K4000-1
5. lung	Clontech	Human Total RNA Panel I, K4000-1
6. trachea	Clontech	Human Total RNA Panel I, K4000-1
7. bone marrow	Clontech	Human Total RNA Panel II, K4001-1
8. colon	Clontech	Human Total RNA Panel II, K4001-1
9. small intestine	Clontech	Human Total RNA Panel II, K4001-1
10. spleen	Clontech	Human Total RNA Panel II, K4001-1
11. stomach	Clontech	Human Total RNA Panel II, K4001-1
12. thymus	Clontech	Human Total RNA Panel II, K4001-1
13. mammary gland	Clontech	Human Total RNA Panel III, K4002-1
14. skeletal muscle	Clontech	Human Total RNA Panel III, K4002-1
15. prostate	Clontech	Human Total RNA Panel III, K4002-1
16. testis	Clontech	Human Total RNA Panel III, K4002-1
17. uterus	Clontech	Human Total RNA Panel III, K4002-1
18. cerebellum	Clontech	Human Total RNA Panel IV, K4003-1
19. fetal brain	Clontech	Human Total RNA Panel IV, K4003-1
20. fetal liver	Clontech	Human Total RNA Panel IV, K4003-1
21. spinal cord	Clontech	Human Total RNA Panel IV, K4003-1
22. placenta	Clontech	Human Total RNA Panel IV, K4003-1
23. adrenal gland	Clontech	Human Total RNA Panel V, K4004-1
24. pancreas	Clontech	Human Total RNA Panel V, K4004-1
25. salivary gland	Clontech	Human Total RNA Panel V, K4004-1
26. thyroid	Clontech	Human Total RNA Panel V, K4004-1

Table 2: **Blood/lung-screen tissues**

Tissue	Supplier	Panel name and catalog number
1. lymph node	Clontech	Human Immune System MTC Panel, K1426-1
2. peripheral blood leukocytes	Clontech	Human Immune System MTC Panel, K1426-1
3. tonsil	Clontech	Human Immune System MTC Panel, K1426-1
4. peripheral blood mononuclear cells	Clontech	Human Blood Fractions MTC Panel, K1428-1
5. peripheral blood mono nuclear cells - activated	Clontech	Human Blood Fractions MTC Panel, K1428-1
6. T-cell (CD8+)	Clontech	Human Blood Fractions MTC Panel, K1428-1
7. T-cell (CD8+) - activated	Clontech	Human Blood Fractions MTC Panel, K1428-1
8. T-cell (CD4+)	Clontech	Human Blood Fractions MTC Panel, K1428-1
9. T-cell (CD4+) - activated	Clontech	Human Blood Fractions MTC Panel, K1428-1
10. B-cell (CD19+)	Clontech	Human Blood Fractions MTC Panel, K1428-1
11. B-cell (CD19+) - activated	Clontech	Human Blood Fractions MTC Panel, K1428-1
12. Monocytes (CD14+)	Clontech	Human Blood Fractions MTC Panel, K1428-1
13. Th1 clone	In-house	
14. Th2 clone	In-house	
15. neutrophil	In-house	
16. neutrophil	In-house	
17. Normal Bronchial/ Tracheal Epithelial Cells	In-house	
18. Normal Bronchial/ Tracheal smooth muscle cell	In-house	
19. Normal lung fibroblast	In-house	
20. Microvascular Endothelial cell	In-house	
21. U937	In-house	
22. RAMOS	In-house	
23. Jurkat	In-house	
24. HelaS3	In-house	
25. IMR-90	In-house	
26. HEK293	In-house	

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CLAIMS

1. An isolated polynucleotide encoding a sphingosine kinase-like protein polypeptide and being selected from the group consisting of:

a) a polynucleotide encoding a sphingosine kinase-like protein polypeptide comprising an amino acid sequence selected form the group consisting of:

amino acid sequences which are at least about 50% identical to the amino acid sequence shown in SEQ ID NO: 2;
the amino acid sequence shown in SEQ ID NO: 2;
amino acid sequences which are at least about 50% identical to the amino acid sequence shown in SEQ ID NO: 10;
the amino acid sequence shown in SEQ ID NO: 10;
amino acid sequences which are at least about 50% identical to the amino acid sequence shown in SEQ ID NO: 11; and
the amino acid sequence shown in SEQ ID NO: 11;

b) a polynucleotide comprising the sequence of SEQ ID NO: 1 or 9;

c) a polynucleotide which hybridizes under stringent conditions to a polynucleotide specified in (a) and (b);

d) a polynucleotide the sequence of which deviates from the polynucleotide sequences specified in (a) to (c) due to the degeneration of the genetic code; and

e) a polynucleotide which represents a fragment, derivative or allelic variation of a polynucleotide sequence specified in (a to (d).

2. An expression vector containing any polynucleotide of claim 1.

3. A host cell containing the expression vector of claim 2.

4. A substantially purified sphingosine kinase-like protein polypeptide encoded by a polynucleotide of claim 1.

5. A method for producing a sphingosine kinase-like protein polypeptide, wherein the method comprises the following steps:

a) culturing the host cell of claim 3 under conditions suitable for the expression of the sphingosine kinase-like protein polypeptide; and

b) recovering the sphingosine kinase-like protein polypeptide from the host cell culture.

6. A method for detection of a polynucleotide encoding a sphingosine kinase-like protein polypeptide in a biological sample comprising the following steps:

a) hybridizing any polynucleotide of claim 1 to a nucleic acid material of a biological sample, thereby forming a hybridization complex; and

b) detecting said hybridization complex.

7. The method of claim 6, wherein before hybridization, the nucleic acid material of the biological sample is amplified.

8. A method for the detection of a polynucleotide of claim 1 or a sphingosine kinase-like protein polypeptide of claim 4 comprising the steps of:

contacting a biological sample with a reagent which specifically interacts with the polynucleotide or the sphingosine kinase-like protein polypeptide.

9. A diagnostic kit for conducting the method of any one of claims 6 to 8.

10. A method of screening for agents which decrease the activity of a sphingosine kinase-like protein, comprising the steps of:

contacting a test compound with any sphingosine kinase-like protein polypeptide encoded by any polynucleotide of claim1;

detecting binding of the test compound to the sphingosine kinase-like protein polypeptide, wherein a test compound which binds to the polypeptide is identified as a potential therapeutic agent for decreasing the activity of a sphingosine kinase-like protein.

11. A method of screening for agents which regulate the activity of a sphingosine kinase-like protein, comprising the steps of:

contacting a test compound with a sphingosine kinase-like protein polypeptide encoded by any polynucleotide of claim 1; and
detecting a sphingosine kinase-like protein activity of the polypeptide, wherein a test compound which increases the sphingosine kinase-like protein activity is identified as a potential therapeutic agent for increasing the activity of the sphingosine kinase-like protein, and wherein a test compound which decreases the sphingosine kinase-like protein activity of the polypeptide is identified as a potential therapeutic agent for decreasing the activity of the sphingosine kinase-like protein.

12. A method of screening for agents which decrease the activity of a sphingosine kinase-like protein, comprising the steps of:

contacting a test compound with any polynucleotide of claim 1 and detecting binding of the test compound to the polynucleotide, wherein a test compound which binds to the polynucleotide is identified as a potential therapeutic agent for decreasing the activity of sphingosine kinase-like protein.

13. A method of reducing the activity of sphingosine kinase-like protein, comprising the steps of:

contacting a cell with a reagent which specifically binds to any polynucleotide of claim 1 or any sphingosine kinase-like protein polypeptide of claim 4, whereby the activity of sphingosine kinase-like protein is reduced.

14. A reagent that modulates the activity of a sphingosine kinase-like protein polypeptide or a polynucleotide wherein said reagent is identified by the method of any of the claim 10 to 12.

15. A pharmaceutical composition, comprising:

the expression vector of claim 2 or the reagent of claim 14 and a pharmaceutically acceptable carrier.

16. Use of the expression vector of claim 2 or the reagent of claim 14 to produce a medicament for modulating the activity of a sphingosine kinase-like protein in a disease.

17. Use of claim 16 wherein the disease is cancer, asthma, allergy, an autoimmune disease or a central or peripheral nervous system disorder.

18. A cDNA encoding a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 2, 10 or 11.

19. The cDNA of claim 18 which comprises SEQ ID NO: 1 or 9.

20. The cDNA of claim 18 which consists of SEQ ID NO: 1 or 9.

21. An expression vector comprising a polynucleotide which encodes a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 2, 10 or 11.

22. The expression vector of claim 21 wherein the polynucleotide consists of SEQ ID NO: 1 or 9.

23. A host cell comprising an expression vector which encodes a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 2, 10 or 11.

24. The host cell of claim 23 wherein the polynucleotide consists of SEQ ID NO: 1 or 9.

25. A purified polypeptide comprising the amino acid sequence shown in SEQ ID NO: 2, 10 or 11.

26. The purified polypeptide of claim 25 which consists of the amino acid sequence shown in SEQ ID NO: 2, 10 or 11.

27. A fusion protein comprising a polypeptide having the amino acid sequence shown in SEQ ID NO: 2, 10 or 11.

28. A method of producing a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 2, 10 or 11, comprising the steps of:

culturing a host cell comprising an expression vector which encodes the polypeptide under conditions whereby the polypeptide is expressed; and

isolating the polypeptide.

29. The method of claim 28 wherein the expression vector comprises SEQ ID NO: 1 or 9.

30. A method of detecting a coding sequence for a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 2, 10 or 11, comprising the steps of:

hybridizing a polynucleotide comprising 11 contiguous nucleotides of SEQ ID NO: 1 or 9 to nucleic acid material of a biological sample, thereby forming a hybridization complex; and

detecting the hybridization complex.

31. The method of claim 30 further comprising the step of amplifying the nucleic acid material before the step of hybridizing.

32. A kit for detecting a coding sequence for a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 2, 10 or 11, comprising:

a polynucleotide comprising 11 contiguous nucleotides of SEQ ID NO: 1 or 9; and
instructions for the method of claim 30.

33. A method of detecting a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 2, 10 or 11, comprising the steps of:

contacting a biological sample with a reagent that specifically binds to the polypeptide to form a reagent-polypeptide complex; and

detecting the reagent-polypeptide complex.

34. The method of claim 33 wherein the reagent is an antibody.

35. A kit for detecting a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 2, 10 or 11, comprising:

an antibody which specifically binds to the polypeptide; and

instructions for the method of claim 33.

36. A method of screening for agents which can modulate the activity of a human sphingosine kinase-like protein, comprising the steps of:

contacting a test compound with a polypeptide comprising an amino acid sequence selected from the group consisting of: (1) amino acid sequences which are at least about 50% identical to the amino acid sequence shown in SEQ ID NO: 2, 10 or 11 and (2) the amino acid sequence shown in SEQ ID NO: 2, 10 or 11; and

detecting binding of the test compound to the polypeptide, wherein a test compound which binds to the polypeptide is identified as a potential agent for regulating activity of the human sphingosine kinase-like protein.

37. The method of claim 36 wherein the step of contacting is in a cell.

38. The method of claim 36 wherein the cell is *in vitro*.

39. The method of claim 36 wherein the step of contacting is in a cell-free system.

40. The method of claim 36 wherein the polypeptide comprises a detectable label.

41. The method of claim 36 wherein the test compound comprises a detectable label.

42. The method of claim 36 wherein the test compound displaces a labeled ligand which is bound to the polypeptide.

43. The method of claim 36 wherein the polypeptide is bound to a solid support.

44. The method of claim 36 wherein the test compound is bound to a solid support.

45. A method of screening for agents which modulate an activity of a human sphingosine kinase-like protein, comprising the steps of:

contacting a test compound with a polypeptide comprising an amino acid sequence selected from the group consisting of: (1) amino acid sequences which are at least about 50% identical to the amino acid sequence shown in SEQ ID NO: 2, 10 or 11 and (2) the amino acid sequence shown in SEQ ID NO: 2, 10 or 11; and

detecting an activity of the polypeptide, wherein a test compound which increases the activity of the polypeptide is identified as a potential agent for increasing the activity of the human sphingosine kinase-like protein, and wherein a test compound which decreases the activity of the polypeptide is identified as a potential agent for decreasing the activity of the human sphingosine kinase-like protein.

46. The method of claim 45 wherein the step of contacting is in a cell.

47. The method of claim 45 wherein the cell is *in vitro*.

48. The method of claim 45 wherein the step of contacting is in a cell-free system.

49. A method of screening for agents which modulate an activity of a human sphingosine kinase-like protein, comprising the steps of:

contacting a test compound with a product encoded by a polynucleotide which comprises the nucleotide sequence shown in SEQ ID NO: 1 or 9; and

detecting binding of the test compound to the product, wherein a test compound which binds to the product is identified as a potential agent for regulating the activity of the human sphingosine kinase-like protein.

50. The method of claim 49 wherein the product is a polypeptide.

51. The method of claim 49 wherein the product is RNA.

52. A method of reducing activity of a human sphingosine kinase-like protein, comprising the step of:

contacting a cell with a reagent which specifically binds to a product encoded by a polynucleotide comprising the nucleotide sequence shown in SEQ ID NO: 1 or 9, whereby the activity of a human sphingosine kinase-like protein is reduced.

53. The method of claim 52 wherein the product is a polypeptide.

54. The method of claim 53 wherein the reagent is an antibody.

55. The method of claim 52 wherein the product is RNA.

56. The method of claim 55 wherein the reagent is an antisense oligonucleotide.

57. The method of claim 56 wherein the reagent is a ribozyme.

58. The method of claim 52 wherein the cell is *in vitro*.

59. The method of claim 52 wherein the cell is *in vivo*.

60. A pharmaceutical composition, comprising:

a reagent which specifically binds to a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 2, 10 or 11; and

a pharmaceutically acceptable carrier.

61. The pharmaceutical composition of claim 60 wherein the reagent is an antibody.

62. A pharmaceutical composition, comprising:

a reagent which specifically binds to a product of a polynucleotide comprising the nucleotide sequence shown in SEQ ID NO: 1 or 9; and

a pharmaceutically acceptable carrier.

63. The pharmaceutical composition of claim 62 wherein the reagent is a ribozyme.

64. The pharmaceutical composition of claim 62 wherein the reagent is an antisense oligonucleotide.

65. The pharmaceutical composition of claim 62 wherein the reagent is an antibody.

66. A pharmaceutical composition, comprising:

an expression vector encoding a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 2, 10 or 11; and

a pharmaceutically acceptable carrier.

67. The pharmaceutical composition of claim 66 wherein the expression vector comprises SEQ ID NO: 1 or 9.

68. A method of treating a sphingosine kinase-like protein disfunction related disease, wherein the disease is selected from cancer, asthma, allergy, an autoimmune disease or a central or peripheral nervous system disorder comprising the step of:

administering to a patient in need thereof a therapeutically effective dose of a reagent that modulates a function of a human sphingosine kinase-like protein, whereby symptoms of the sphingosine kinase-like protein disfunction related disease are ameliorated.

69. The method of claim 68 wherein the reagent is identified by the method of claim 36.

70. The method of claim 68 wherein the reagent is identified by the method of claim 45.

71. The method of claim 68 wherein the reagent is identified by the method of claim 49.

Fig. 1

accaaagcatttactggtatttatcaacccgtttggaggaaaaggacaag
gcaagcggatatatgaaagaaaagtggcaccactgttcaccttagcctcc
atcaccactgacatcatcggtaacaaattctatgttaactatgtagaagt
aattactgaacatgctaatcaggccaaggagactctgtatgagattaaca
tagacaaatacgacggcatcgtctgtgtcggcggagatggtatgttcagc
gaggtgctgcacggtctgattgggaggacgcagaggagcgccggggtcga
ccagaaccacccccgggctgtgctggtccccagtagcctccggattggaa
tcattcccgcagggtcaacggactgcgtgtgttactccaccgtgggcacc
agcgacgcagaaacctcggcgctgcatatcgttgttggggactcgctggc
catggatgtgtcctcagtccaccacaacagcacactccttcgctactccg
tgtccctgctgggctacggcttctacggggacatcatcaaggacagtgag
aagaaacggtggttgggtcttgccagatacgacttttcaggtttaaagac
cttcctctcccaccactgctatgaagggacagtgtccttcctccctgcac
aacacacggtgggatctccaagggataggaagccctgccgggcaggatgc
tttgtttgcaggcaaagcaagcagcagctggaggaggagcagaagaaagc
actgtatggtttggaagctgcggaggacgtggaggagtggcaagtcgtct
gtgggaagtttctggccatcaatgccacaaacatgtcctgtgcttgtcgc
cggagccccaggggcctctccccggctgcccacttgggagacgggtcttc
tgacctcatcctcatccggaaatgctccaggttcaattttctgagatttc
tcatcaggcacaccaaccagcaggaccag

Fig. 2

PKHLLVFINP FGGKGQGKRI YERKVAPLFT LASITTDIIG
NKFYVNYVEV ITEHANQAKE TLYEINIDKY DGIVCVGGDG
MFSEVLHGLI GRTQRSAGVD QNHPRAVLVP SSLRIGIIPA
GSTDCVCYST VGTSDAETSA LHIVVGDSLA MDVSSVHHNS
TLLRYSVSLL GYGFYGDIIK DSEKKRWLGL ARYDFSGLKT
FLSHHCYEGT VSFLPAQHTV GSPRDRKPCR AGCFVCRQSK
QQLEEEQKKA LYGLEAAEDV EEWQVVCGKF LAINATNMSC
ACRRSPRGLS PAAHLGDGSS DLILIRKCSR FNFLRFLIRH TNQQDQ

Fig. 3

MAPPPPPLAASTPLLHGEFGSYPARGPRFALTLTSQALHIQRLRPKPEARP
RGGLVPLAEVSGCCTLRSRSPSDSAAYFCIYTYPRGRRGARRRATRTFRAD
GAATYEENRAEAQRWATALTCLLRGLPLPGDGEITPDLLPRPPRLLLLVNP
FGGRGLAWQWCKNHVLPMISEAGLSFNLIQTERQNHARELVQGLSLSEWDG
IVTVSGDGLLHEVLNGLLDRPDWEEAVKMPVGILPCGSGNALAGAVNQHGG
FEPALGLDLLLNCSLLLCRGGGHPLDLLSVTLASGSRCFSFLSVAWGFVSD
VDIQSERFRALGSARFTLGTVLGLATLHTYRGRLSYLPATVEPASPTPAHS
LPRAKSELTLTPDPAPPMAHSPLHRSVSDLPLPLPQPALASPGSPEPLPIL
SLNGGGPELAGDWGGAGDAPLSPDPLLSSPPGSPKAALHSPVSEGAPVIPP
SSGLPLPTPDARVGASTCGPPDHLLPPLGTPLPPDWVTLEGDFVLMLAISP
SHLGADLVAAPHARFDDGLVHLCWVRSGISRAALLRLFLAMERGSHFSLGC
PQLGYAAARAFRLEPLTPRGVLTVDGEQVEYGPLQAQMHPGIGTLLTGPPG
CPGREP

Fig. 4

cacgaggggtatgttcagcgaggtgctgcacggtctgattgggaggacgca
gaggagcgccggggtcgaccagaaccacccccgggctgtgctggtccccag
tagcctccggattggaatcattcccgcagggtcaacggactgcgtgtgtta
ctccaccgtgggcaccagcgacgcagaaacctcggcgctgcatatcgttgt
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ggcaagatgctttgg

Fig. 5

tcaccactgacatcatcgttactgaacatgctantcaggccanggagactc
tgtatgagattaacatagacaaatacgacggcatcgtctgtgtcggcggag
atggtatgttcagcgaggtgctgcacggtctgattgggaggacgcagagga
gcgccggggtcgaccagaaccacccccgggctgtgctggtccccagtagcc
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ccgtgggcancagcgacgcagaaacctcggcgctgcatatcgttgttgggg
actcgctggccatggatgtgtcc

Fig. 6

gtttaaagaccttcctctcccaccactgctatgaagggacagtgtccttcc
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caggatgctttgtttgcaggcaaagcaagcagcagctggaggaggagcaga
agaaagcactgtat

Fig. 7

gggactcgctggccatggatgtgtcctcagtccaccacaacagcacactcc
ttcgctactccgtgtccctgctgggctacggcttctacggggacatcatca
aggacagtgagaagaaacggtggttgggtcttgccagatacgacttttcag

Fig. 8

Cacgaggccgctaacggtccggcgcccctcggcgtccgcgcgcccccagcc
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cccgcgcgggctctgctgcgctggtggcggagcccggggcccggagccggc
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agaccaaagcatttactggtatttatcaacccgtttggaggaaaaggacaa
ggcaagcggatatatgaaagaaaagtggcaccactgttca

Fig. 9

CACGAGGCCGCTAACGGTCCGGCGCCCCTCGGCGTCCGCGCGCCCCCAGC
CTGGCGGACGAGCCCGGCGGCGGAGATGGGGGCGACGGGGGCGGCGGAGC
CGCTGCAATCCGTGCTGTGGGTGAAGCAGCAGCGCTGCGCCGTGAGCCTG
GAGCCCGCGCGGGCTCTGCTGCGCTGGTGGCGGAGCCCGGGGCCCGGAGC
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GCCAAGGAGACTCTGTATGAGATTAACATAGACAAATACGACGGCATCGT
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GGAGGACGCAGAGGAGCGCCGGGGTCGACCAGAACCACCCCCGGGCTGTG
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GAAGGGACAGTGTCCTTCCTCCCTGCACAACACACGGTGGGATCTCCAAG
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AGCAGCTGGAGGAGGAGCAGAAGAAAGCACTGTATGGTTTGGAAGCTGCG
GAGGACGTGGAGGAGTGGCAAGTCGTCTGTGGGAAGTTTCTGGCCATCAA
TGCCACAAACATGTCCTGTGCTTGTCGCCGGAGCCCCAGGGGCCTCTCCC
CGGCTGCCCACTTGGGAGACGGGTCTTCTGACCTCATCCTCATCCGGAAA
TGCTCCAGGTTCAATTTTCTGAGATTTCTCATCAGGCACACCAACCAGCA
GGACCAGTTTGACTTCACTTTTGTTGAAGTTTATCGCGTCAAGAAATTCC
AGTTTACGTCGAAGCACATGGAGGATGAGGACAGCGACCTCAAGGAGGGG
GGGAAGAAGCGCTTTGGGCACATTTGCAGCAGCCACCCCTCCTGCTGCTG
CACCGTCTCCAACAGCTCCTGGAACTGCGACGGGGAGGTCCTGCACAGCC
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GCTTACTCTCAGCGGATGCAGCTGCTCACTTGGGGGCACTGGCCTCTTAG
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TCTGGGCCTGAGGTAGCAGTGGGTCACTTTGATCCACTTTGCAGGACTTA
TTCTGTAACGGTTTGTGGCCAAGTTTTGGGAAGTGGTTGATTCTCTTTGC
CTTCATTTCACCTTCCTCTTCGTTTACGGTTAGGACATCGCTGCTTGATC

Fig. 9 (continued)

CTTACAATACTGTGCAACTGCAATGCAACGTGGCCCTGCTTCAGGTGATC
CGCGGGAGGGGCCTCCACGCCAGCGCCGGGAAGGCTGCTGGGGCCTCCAC
ACCTGCCTCATCACGGCGGCGAGGCTACGACAATCCGGCTGGGAGCATGA
CCTTGGCGTCTGTTCTGGGAGCACAGATGATAAGCTCTGGAAGCTGGCAG
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CTTTATATCGACGCGAAGTGCTTAACACAGCCGGGCTTGGGGGCAGTCAG
GAGGAAGCTGGCCATCCGTGGAGGAGGGGCCGGTCCTGGACTCCCGCAGG
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CCAGAGGATTCTTCCCGGAAGGCATTCCTGCCGCGCTCCCCGGGGCACCC
CTCAATTGTGTACTACGTCCTTGTTTAGTGTGTATCCGTGCCCACGTAGA
TGATGTCTGTAACGTAGTTTTGTTTGAAATATGAGAATATGCGGCTTAAA
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TGGAAAGAAACCAAAGATTTAAGACGGGCTGCTCTTCCAGACTGGCTGTG
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GTTTCCCCAAAAGGTTCTTCTAAGCCTTTATTGTCCCTGGTAAATGTTTC
CCGGCTGGGCGCGGTGGCTCACGCCTGTAATCCCAGCACTTTGGGAGGCC
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CCAGCCACCTGCTGACATTGAGAATCTCAGACCTCGGGACTGCTGTTGCG
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TTAGAAAGGCTAGTTTTAGCTTCCTTTGTGGCTCCCTGGTGCAAAACAAT
TAGCAGTTATGCAATGGACCTGATTCTAGTTTATTCTAATTAAGAAGTGA
GGCCGAGTTTGACTTCGTTCCTGAATACAATCTTGAGTAACTGGGAAAGT
CTGAGTGAAAGGATGGCCTCATTCTCTTTCTAATCTTGCTGGTTTCAAGA
TTAGAAAATGGCATTATTTGATCTGAAATGTTTGAGAAGACACGAATAAA

GTTACTTGGGCAG

Fig. 10

MGATGAAEPLQSVLWVKQQRCAVSLEPARALLRWWRSPGPGAGAPGADAC
SVPVSEIIAVEETDVHGKHQGSGKWQKMEKPYAFTVHCVKRARRHRWKWA
QVTFWCPEEQLCHLWLQTLREMLEKLTSRPKHLLVFINPFGGKGQGKRIY
ERKVAPLFTLASITTDIIVTEHANQAKETLYEINIDKYDGIVCVGGDGMF
SEVLHGLIGRTQRSAGVDQNHPRAVLVPSSLRIGIIPAGSTDCVCYSTVG
TSDAETSALHIVVGDSLAMDVSSVHHNSTLLRYSVSLLGYGFYGDIIKDS
EKKRWLGLARYDFSGLKTFLSHHCYEGTVSFLPAQHTVGSPRDRKPCRAG
CFVCRQSKQQLEEEQKKALYGLEAAEDVEEWQVVCGKFLAINATNMSCAC
RRSPRGLSPAAHLGDGSSDLILIRKCSRFNFLRFLIRHTNQQDQFDFTFV
EVYRVKKFQFTSKHMEDEDSDLKEGGKKRFGHICSSHPSCCCTVSNSSWN
CDGEVLHSPAIEVRVHCQLVRLFARGIEENPKPDSHS

Fig. 11

HEAANGPAPLGVRAPPAWRTSPAAEMGATGAAEPLQSVLWVKQQRCAVSL
EPARALLRWWRSPGPGAGAPGADACSVPVSEIIAVEETDVHGKHQGSGKW
QKMEKPYAFTVHCVKRARRHRWKWAQVTFWCPEEQLCHLWLQTLREMLEK
LTSRPKHLLVFINPFGGKGQGKRIYERKVAPLFTLASITTDIIVTEHANQ
AKETLYEINIDKYDGIVCVGGDGMFSEVLHGLIGRTQRSAGVDQNHPRAV
LVPSSLRIGIIPAGSTDCVCYSTVGTSDAETSALHIVVGDSLAMDVSSVH
HNSTLLRYSVSLLGYGFYGDIIKDSEKKRWLGLARYDFSGLKTFLSHHCY
EGTVSFLPAQHTVGSPRDRKPCRAGCFVCRQSKQQLEEEQKKALYGLEAA
EDVEEWQVVCGKFLAINATNMSCACRRSPRGLSPAAHLGDGSSDLILIRK
CSRFNFLRFLIRHTNQQDQFDFTFVEVYRVKKFQFTSKHMEDEDSDLKEG
GKKRFGHICSSHPSCCCTVSNSSWNCDGEVLHSPAIEVRVHCQLVRLFAR
GIEENPKPDSHS

Fig. 12

BLASTP - alignment of Sphigosine_kinase against tremblnew|AF245447|AF245447_1 product: "sphingosine kinase type 2 isoform"; Homo sapiens sphingosine kinase type 2 isoform mRNA, complete cds. //:gp|AF245447|8248285 product: "sphingosine kinase type 2 isoform"; Homo sapiens sphingosine kinase type 2 isoform mRNA, complete cds. //:gpnew|AF245447|8248285 product: "sphingosine kinase type 2 isoform"; Homo sapiens sphingosine kinase type 2 isoform mRNA, complete cds.

This hit is scoring at : 1e-17 (expectation value) Alignment length (overlap) : 232 Identities : 28 % Scoring matrix : BLOSUM62 (used to infer consensus pattern) Database searched : nrdb

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Q: 1     PKHLLVFINPFGGKGQGKRIYERKVAPLFTLASITTDIIGNKFYVNYVEVITEHANQAKE
         P..LL:..:NPFGG:G . : :..V.P:.: A.:: ::I             TE..N.A:E
H: 144   PPRLLLLVNPFGGRGLAWQWCKNHVLPMISEAGLSFNLIQ----------TERQNHARE

         TLYEINIDKYDGIVCVGGDGMFSEVLHGLIGRTQRSAGVDQNHPRAVLVPSSLRIGIIPA
         .:. :::.::DGIV.V.GDG:..EVL:GL:.R .: ..       AV :P    :GI:P.
         LVQGLSLSEWDGIVTVSGDGLLHEVLNGLLDR--------PDWEEAVKMP----VGILPC

         GSTDCVCYS---------TVGTSDAETSALHIVVGDSLAMDVSSVHHNSTLLRYSVSLLG
         GS :.:. : .:G.. . ..:L :.          G.. .:D: SV   S    :S. :.
         GSGNALAGAVNQHGGFEPALGLDLLLNCSLLLCRGGGHPLDLLSVTLASGSRCFSFLSVA

         YGFYGDIIKDSEKKRWLGLARYDFSGLKTFLSHHCYEGTVSFLPAQHTVGSP 223
         :GF..D:  .SE: R LG AR:... :  ..: H.Y.G.:S:LPA. . .SP
         WGFVSDVDIQSERFRALGSARFTLGTVLGLATLHTYRGRLSYLPATVEPASP 352
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Diacylglycerol kinase catalytic domain in bold

Fig. 13

PKHLLVFINP FGGKGQGKRI YERKVAPLFT LASITTDIIG NKFYVNYVEV ITEHANQAKE
TLYEINIDKY DGIVCVGGDG MFSEVLHGLI GRTQRSAGVD QNHPRAVLVP SSLRIGIIPA
GSTDCVCYST VGTSDAETSA LHIVVGDSLA MDVSSVHHNS TLLRYSVSLL GYGFYGDIIK
DSEKKRWLGL ARYDFSGLKT FLSHHCYEGT VSFLPAQHTV GSPRDRKPCR AGCFVCRQSK
QQLEEEQKKA LYGLEAAEDV EEWQVVCGKF LAINATNMSC ACRRSPRGLS PAAHLGDGSS
DLILIRKCSR FNFLRFLIRH TNQQDQ

Diacylglycerol kinase catalytic domain in bold

Fig. 14

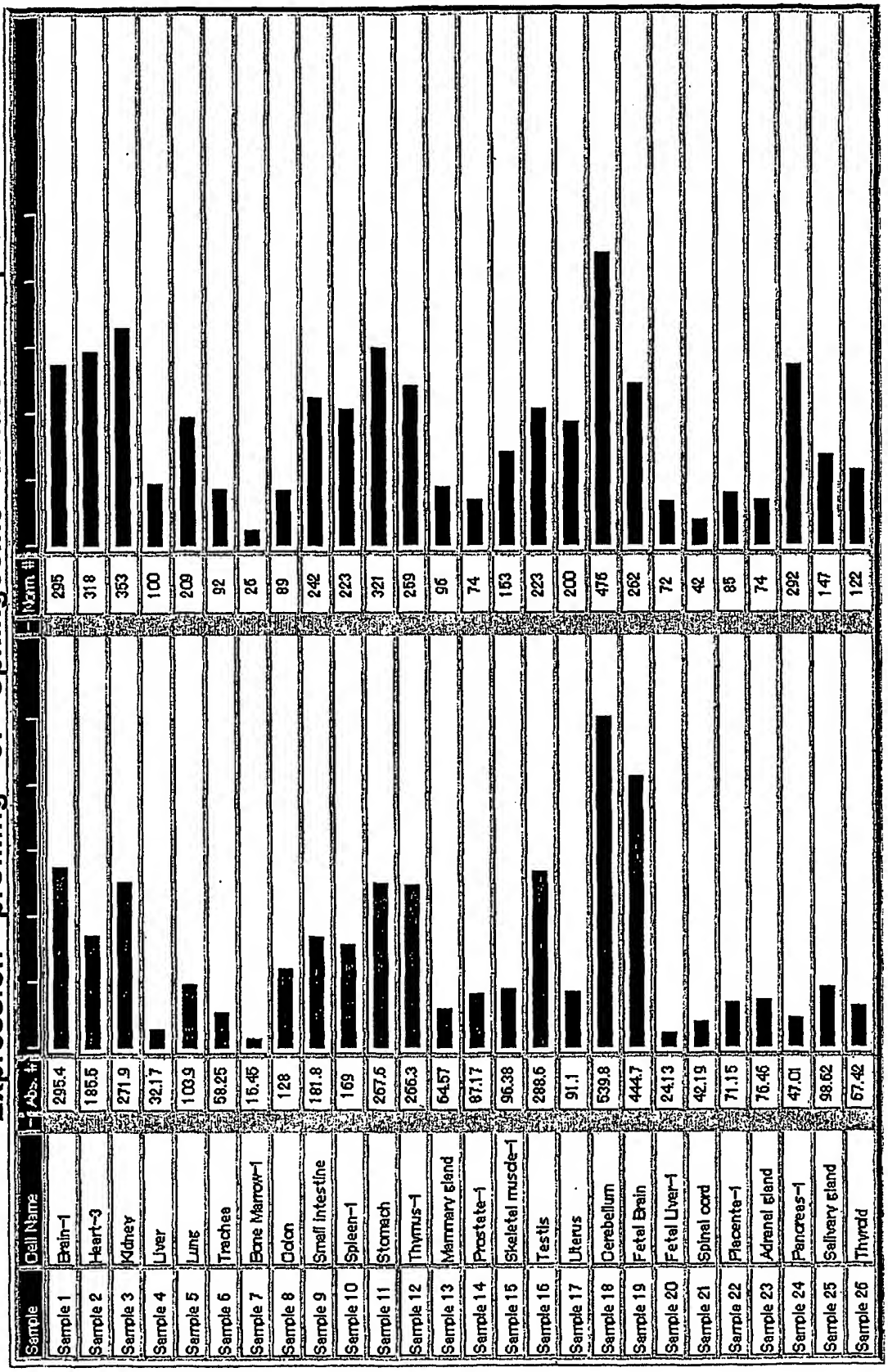
Expression profiling of Sphingosine kinase-like protein
Sample
Cell Name
Abs. #
Norm #
Sample 1 Brain-1 295.4 295
Sample 2 Heart-3 185.5 318
Sample 3 Kidney 271.9 353
Sample 4 Liver 32.17 100
Sample 5 Lung 103.9 209
Sample 6 Trachea 58.25 92
Sample 7 Bone Marrow-1 16.45 26
Sample 8 Colon 128 89
Sample 9 Small intestine 181.8 242
Sample 10 Spleen-1 169 223
Sample 11 Stomach 267.6 321
Sample 12 Thymus-1 206.3 269
Sample 13 Mammary gland 64.57 96
Sample 14 Prostate-1 87.17 74
Sample 15 Skeletal muscle-1 96.38 153
Sample 16 Testis 288.6 223
Sample 17 Uterus 91.1 200
Sample 18 Cerebellum 639.8 476
Sample 19 Fetal Brain 444.7 262
Sample 20 Fetal Liver-1 24.13 72
Sample 21 Spinal cord 42.19 42
Sample 22 Placenta-1 71.15 85
Sample 23 Adrenal gland 76.46 74
Sample 24 Pancreas-1 47.01 292
Sample 25 Salivary gland 98.62 147
Sample 26 Thyroid 67.42 122

Fig. 15

Expression profiling of Sphingosine kinase-like protein mRNA, blood/lung screen

Sample	Cell Name	Abs. #	Norm. #
Sample 1	lymph node	21.51	21
Sample 2	PBL	12.78	9
Sample 3	Tonsil	11.54	58
Sample 4	PBMN	78.21	24
Sample 5	PBMN stimulated	54.03	45
Sample 6	T-cell(CD8+)	612.7	56
Sample 7	T-cell(CD8+)activated	11.58	23
Sample 8	T-cell(CD4+)	348.7	41
Sample 9	T-cell(CD4+) activated	48.88	125
Sample 10	B-cell(CD19+)	59.65	19
Sample 11	B-cell(CD19+) activated	10.55	23
Sample 12	Monocytes(CD14+)	52.88	20
Sample 13	Th1 clone	141.9	16
Sample 14	Th2 clone	3.747	0
Sample 15	neutroA-2	294.1	7
Sample 16	neutroB-2	163.8	7
Sample 17	Normal Bronchial/Tracheal Epithelial Cells	55.63	262
Sample 18	Normal Bronchial/Tracheal smooth muscle cell	42.76	139
Sample 19	Normal lung fibroblast	111.4	75
Sample 20	Microvascular Endothelial cell	630.9	530
Sample 21	U937	53.94	119
Sample 22	RAMOS	53.35	236
Sample 23	Jurkat	58.99	187
Sample 24	HelaS3	155.6	197
Sample 25	IMR-90	172.3	217
Sample 26	HEK293	.712	0

SEQUENCE LISTING

<110> Bayer AG

<120> REGULATION OF HUMAN SPHINGOSINE KINASE-LIKE PROTEIN

<130> LIO160 Foreign Countries

<150> US 60/238,005
<151> 2000-10-06

<150> US 60/XXX,XXX
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Gly Lys Arg Ile Tyr Glu Arg Lys Val Ala Pro Leu Phe Thr Leu Ala
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Ser Ile Thr Thr Asp Ile Ile Gly Asn Lys Phe Tyr Val Asn Tyr Val
        35                  40                  45

Glu Val Ile Thr Glu His Ala Asn Gln Ala Lys Glu Thr Leu Tyr Glu
    50                  55                  60

Ile Asn Ile Asp Lys Tyr Asp Gly Ile Val Cys Val Gly Gly Asp Gly
65                  70                  75                  80

Met Phe Ser Glu Val Leu His Gly Leu Ile Gly Arg Thr Gln Arg Ser
                85                  90                  95

Ala Gly Val Asp Gln Asn His Pro Arg Ala Val Leu Val Pro Ser Ser
            100                 105                 110

Leu Arg Ile Gly Ile Ile Pro Ala Gly Ser Thr Asp Cys Val Cys Tyr
        115                 120                 125

Ser Thr Val Gly Thr Ser Asp Ala Glu Thr Ser Ala Leu His Ile Val
    130                 135                 140

Val Gly Asp Ser Leu Ala Met Asp Val Ser Ser Val His His Asn Ser
145                 150                 155                 160

Thr Leu Leu Arg Tyr Ser Val Ser Leu Leu Gly Tyr Gly Phe Tyr Gly
                165                 170                 175

Asp Ile Ile Lys Asp Ser Glu Lys Lys Arg Trp Leu Gly Leu Ala Arg
            180                 185                 190

Tyr Asp Phe Ser Gly Leu Lys Thr Phe Leu Ser His His Cys Tyr Glu
        195                 200                 205

Gly Thr Val Ser Phe Leu Pro Ala Gln His Thr Val Gly Ser Pro Arg
    210                 215                 220

Asp Arg Lys Pro Cys Arg Ala Gly Cys Phe Val Cys Arg Gln Ser Lys
225                 230                 235                 240
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Gln Gln Leu Glu Glu Glu Gln Lys Lys Ala Leu Tyr Gly Leu Glu Ala
                245                 250                 255

Ala Glu Asp Val Glu Glu Trp Gln Val Val Cys Gly Lys Phe Leu Ala
            260                 265                 270

Ile Asn Ala Thr Asn Met Ser Cys Ala Cys Arg Arg Ser Pro Arg Gly
        275                 280                 285

Leu Ser Pro Ala Ala His Leu Gly Asp Gly Ser Ser Asp Leu Ile Leu
    290                 295                 300

Ile Arg Lys Cys Ser Arg Phe Asn Phe Leu Arg Phe Leu Ile Arg His
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Thr Asn Gln Gln Asp Gln
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            20                  25                  30

Leu Thr Ser Gln Ala Leu His Ile Gln Arg Leu Arg Pro Lys Pro Glu
        35                  40                  45

Ala Arg Pro Arg Gly Gly Leu Val Pro Leu Ala Glu Val Ser Gly Cys
    50                  55                  60

Cys Thr Leu Arg Ser Arg Ser Pro Ser Asp Ser Ala Ala Tyr Phe Cys
65                  70                  75                  80

Ile Tyr Thr Tyr Pro Arg Gly Arg Arg Gly Ala Arg Arg Arg Ala Thr
                85                  90                  95

Arg Thr Phe Arg Ala Asp Gly Ala Ala Thr Tyr Glu Glu Asn Arg Ala
            100                 105                 110

Glu Ala Gln Arg Trp Ala Thr Ala Leu Thr Cys Leu Leu Arg Gly Leu
        115                 120                 125
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145                 150                 155                 160

Trp Gln Trp Cys Lys Asn His Val Leu Pro Met Ile Ser Glu Ala Gly
                165                 170                 175

Leu Ser Phe Asn Leu Ile Gln Thr Glu Arg Gln Asn His Ala Arg Glu
            180                 185                 190

Leu Val Gln Gly Leu Ser Leu Ser Glu Trp Asp Gly Ile Val Thr Val
        195                 200                 205

Ser Gly Asp Gly Leu Leu His Glu Val Leu Asn Gly Leu Leu Asp Arg
    210                 215                 220

Pro Asp Trp Glu Glu Ala Val Lys Met Pro Val Gly Ile Leu Pro Cys
225                 230                 235                 240

Gly Ser Gly Asn Ala Leu Ala Gly Ala Val Asn Gln His Gly Gly Phe
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Glu Pro Ala Leu Gly Leu Asp Leu Leu Leu Asn Cys Ser Leu Leu Leu
            260                 265                 270

Cys Arg Gly Gly Gly His Pro Leu Asp Leu Leu Ser Val Thr Leu Ala
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Ser Gly Ser Arg Cys Phe Ser Phe Leu Ser Val Ala Trp Gly Phe Val
    290                 295                 300

Ser Asp Val Asp Ile Gln Ser Glu Arg Phe Arg Ala Leu Gly Ser Ala
305                 310                 315                 320

Arg Phe Thr Leu Gly Thr Val Leu Gly Leu Ala Thr Leu His Thr Tyr
                325                 330                 335

Arg Gly Arg Leu Ser Tyr Leu Pro Ala Thr Val Glu Pro Ala Ser Pro
            340                 345                 350

Thr Pro Ala His Ser Leu Pro Arg Ala Lys Ser Glu Leu Thr Leu Thr
        355                 360                 365

Pro Asp Pro Ala Pro Pro Met Ala His Ser Pro Leu His Arg Ser Val
    370                 375                 380
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Ser Asp Leu Pro Leu Pro Leu Pro Gln Pro Ala Leu Ala Ser Pro Gly
385                 390                 395                 400

Ser Pro Glu Pro Leu Pro Ile Leu Ser Leu Asn Gly Gly Gly Pro Glu
                405                 410                 415

Leu Ala Gly Asp Trp Gly Gly Ala Gly Asp Ala Pro Leu Ser Pro Asp
            420                 425                 430

Pro Leu Leu Ser Ser Pro Pro Gly Ser Pro Lys Ala Ala Leu His Ser
        435                 440                 445

Pro Val Ser Glu Gly Ala Pro Val Ile Pro Pro Ser Ser Gly Leu Pro
    450                 455                 460

Leu Pro Thr Pro Asp Ala Arg Val Gly Ala Ser Thr Cys Gly Pro Pro
465                 470                 475                 480

Asp His Leu Leu Pro Pro Leu Gly Thr Pro Leu Pro Pro Asp Trp Val
                485                 490                 495

Thr Leu Glu Gly Asp Phe Val Leu Met Leu Ala Ile Ser Pro Ser His
            500                 505                 510

Leu Gly Ala Asp Leu Val Ala Ala Pro His Ala Arg Phe Asp Asp Gly
        515                 520                 525

Leu Val His Leu Cys Trp Val Arg Ser Gly Ile Ser Arg Ala Ala Leu
    530                 535                 540

Leu Arg Leu Phe Leu Ala Met Glu Arg Gly Ser His Phe Ser Leu Gly
545                 550                 555                 560

Cys Pro Gln Leu Gly Tyr Ala Ala Ala Arg Ala Phe Arg Leu Glu Pro
                565                 570                 575

Leu Thr Pro Arg Gly Val Leu Thr Val Asp Gly Glu Gln Val Glu Tyr
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Gly Pro Pro Gly Cys Pro Gly Arg Glu Pro
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cattcccgca gggtcaacgg actgcgtgtg ttactccacc gtgggcacca gcgacgcaga 180

aacctcggcg ctgcatatcg ttgttgggga ctcgctggcc atggatgtgt cctcagtcca 240

ccacaacagc acactccttc gctactccgt gtccctgctg ggctacggct tctacgggga 300

catcatcaag gacagtgaga agaaacggtg gttgggtctt gccagatacg acttttcagg 360

tttaaagacc ttcctctccc accactgcta tgaagggaca gtgtccttcc tccctgcaca 420

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tgctgcacgg tctgattggg aggacgcaga ggagcgccgg ggtcgaccag aaccaccccc 180

gggctgtgct ggtccccagt agcctccgga ttggaatcat tcccgcaggt caaacggact 240

gcgtgtntta ctccaccgtg ggcancagcg acgcagaaac ctcggcgctg catatcgttg 300

ttggggactc gctggccatg gatgtgtcc 329

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aacacacggt gggatctcca agggatagga agccctgccg ggcaggatgc tttgtttgca 120

ggcaaagcaa gcagcagctg gaggaggagc agaagaaagc actgtat 167

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ccgtgtccct gctgggctac ggcttctacg gggacatcat caaggacagt gagaagaaac 120

ggtggttggg tcttgccaga tacgactttt cag 153

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gtgaagcagc agcgctgcgc cgtgagcctg gagcccgcgc gggctctgct gcgctggtgg 180

cggagcccgg ggcccggagc cggcgccccc ggcgcggatg cctgctctgt gcctgtatct 240

gagatcatcg ccgttgagga aacagacgtt cacgggaaac atcaaggcag tggaaaatgg 300

cagaaaatgg aaaagcctta cgcttttaca gttcactgtg taaagagagc acgacggcac 360

cgctggaagt gggcgcaggt gactttctgg tgtccagagg agcagctgtg tcacttgtgg 420

ctgcagaccc tgcgggagat gctggagaag ctgacgtcca gaccaaagca tttactggta 480

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ccactgttca 550

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                 Met Gly Ala Thr Gly Ala Ala Glu Pro Leu Gln Ser
                 1               5                  10

gtg ctg tgg gtg aag cag cag cgc tgc gcc gtg agc ctg gag ccc gcg      159
Val Leu Trp Val Lys Gln Gln Arg Cys Ala Val Ser Leu Glu Pro Ala
        15                  20                  25

cgg gct ctg ctg cgc tgg tgg cgg agc ccg ggg ccc gga gcc ggc gcc      207
Arg Ala Leu Leu Arg Trp Trp Arg Ser Pro Gly Pro Gly Ala Gly Ala
    30                  35                  40

ccc ggc gcg gat gcc tgc tct gtg cct gta tct gag atc atc gcc gtt      255
Pro Gly Ala Asp Ala Cys Ser Val Pro Val Ser Glu Ile Ile Ala Val
45                  50                  55                  60

gag gaa aca gac gtt cac ggg aaa cat caa ggc agt gga aaa tgg cag      303
Glu Glu Thr Asp Val His Gly Lys His Gln Gly Ser Gly Lys Trp Gln
                65                  70                  75

aaa atg gaa aag cct tac gct ttt aca gtt cac tgt gta aag aga gca      351
Lys Met Glu Lys Pro Tyr Ala Phe Thr Val His Cys Val Lys Arg Ala
            80                  85                  90

cga cgg cac cgc tgg aag tgg gcg cag gtg act ttc tgg tgt cca gag      399
Arg Arg His Arg Trp Lys Trp Ala Gln Val Thr Phe Trp Cys Pro Glu
        95                  100                 105

gag cag ctg tgt cac ttg tgg ctg cag acc ctg cgg gag atg ctg gag      447
Glu Gln Leu Cys His Leu Trp Leu Gln Thr Leu Arg Glu Met Leu Glu
    110                 115                 120

aag ctg acg tcc aga cca aag cat tta ctg gta ttt atc aac ccg ttt      495
Lys Leu Thr Ser Arg Pro Lys His Leu Leu Val Phe Ile Asn Pro Phe
125                 130                 135                 140

gga gga aaa gga caa ggc aag cgg ata tat gaa aga aaa gtg gca cca      543
Gly Gly Lys Gly Gln Gly Lys Arg Ile Tyr Glu Arg Lys Val Ala Pro
                145                 150                 155

ctg ttc acc tta gcc tcc atc acc act gac atc atc gtt act gaa cat      591
Leu Phe Thr Leu Ala Ser Ile Thr Thr Asp Ile Ile Val Thr Glu His
            160                 165                 170

gct aat cag gcc aag gag act ctg tat gag att aac ata gac aaa tac      639
Ala Asn Gln Ala Lys Glu Thr Leu Tyr Glu Ile Asn Ile Asp Lys Tyr
        175                 180                 185

gac ggc atc gtc tgt gtc ggc gga gat ggt atg ttc agc gag gtg ctg      687
Asp Gly Ile Val Cys Val Gly Gly Asp Gly Met Phe Ser Glu Val Leu
    190                 195                 200

cac ggt ctg att ggg agg acg cag agg agc gcc ggg gtc gac cag aac      735
His Gly Leu Ile Gly Arg Thr Gln Arg Ser Ala Gly Val Asp Gln Asn
205                 210                 215                 220

cac ccc cgg gct gtg ctg gtc ccc agt agc ctc cgg att gga atc att      783
His Pro Arg Ala Val Leu Val Pro Ser Ser Leu Arg Ile Gly Ile Ile
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                225                 230                 235

ccc gca ggg tca acg gac tgc gtg tgt tac tcc acc gtg ggc acc agc      831
Pro Ala Gly Ser Thr Asp Cys Val Cys Tyr Ser Thr Val Gly Thr Ser
            240                 245                 250

gac gca gaa acc tcg gcg ctg cat atc gtt gtt ggg gac tcg ctg gcc      879
Asp Ala Glu Thr Ser Ala Leu His Ile Val Val Gly Asp Ser Leu Ala
        255                 260                 265

atg gat gtg tcc tca gtc cac cac aac agc aca ctc ctt cgc tac tcc      927
Met Asp Val Ser Ser Val His His Asn Ser Thr Leu Leu Arg Tyr Ser
    270                 275                 280

gtg tcc ctg ctg ggc tac ggc ttc tac ggg gac atc atc aag gac agt      975
Val Ser Leu Leu Gly Tyr Gly Phe Tyr Gly Asp Ile Ile Lys Asp Ser
285                 290                 295                 300

gag aag aaa cgg tgg ttg ggt ctt gcc aga tac gac ttt tca ggt tta     1023
Glu Lys Lys Arg Trp Leu Gly Leu Ala Arg Tyr Asp Phe Ser Gly Leu
                305                 310                 315

aag acc ttc ctc tcc cac cac tgc tat gaa ggg aca gtg tcc ttc ctc     1071
Lys Thr Phe Leu Ser His His Cys Tyr Glu Gly Thr Val Ser Phe Leu
            320                 325                 330

cct gca caa cac acg gtg gga tct cca agg gat agg aag ccc tgc cgg     1119
Pro Ala Gln His Thr Val Gly Ser Pro Arg Asp Arg Lys Pro Cys Arg
        335                 340                 345

gca gga tgc ttt gtt tgc agg caa agc aag cag cag ctg gag gag gag     1167
Ala Gly Cys Phe Val Cys Arg Gln Ser Lys Gln Gln Leu Glu Glu Glu
    350                 355                 360

cag aag aaa gca ctg tat ggt ttg gaa gct gcg gag gac gtg gag gag     1215
Gln Lys Lys Ala Leu Tyr Gly Leu Glu Ala Ala Glu Asp Val Glu Glu
365                 370                 375                 380

tgg caa gtc gtc tgt ggg aag ttt ctg gcc atc aat gcc aca aac atg     1263
Trp Gln Val Val Cys Gly Lys Phe Leu Ala Ile Asn Ala Thr Asn Met
                385                 390                 395

tcc tgt gct tgt cgc cgg agc ccc agg ggc ctc tcc ccg gct gcc cac     1311
Ser Cys Ala Cys Arg Arg Ser Pro Arg Gly Leu Ser Pro Ala Ala His
            400                 405                 410

ttg gga gac ggg tct tct gac ctc atc ctc atc cgg aaa tgc tcc agg     1359
Leu Gly Asp Gly Ser Ser Asp Leu Ile Leu Ile Arg Lys Cys Ser Arg
        415                 420                 425

ttc aat ttt ctg aga ttt ctc atc agg cac acc aac cag cag gac cag     1407
Phe Asn Phe Leu Arg Phe Leu Ile Arg His Thr Asn Gln Gln Asp Gln
    430                 435                 440

ttt gac ttc act ttt gtt gaa gtt tat cgc gtc aag aaa ttc cag ttt     1455
Phe Asp Phe Thr Phe Val Glu Val Tyr Arg Val Lys Lys Phe Gln Phe
445                 450                 455                 460

acg tcg aag cac atg gag gat gag gac agc gac ctc aag gag ggg ggg     1503
Thr Ser Lys His Met Glu Asp Glu Asp Ser Asp Leu Lys Glu Gly Gly
                465                 470                 475

aag aag cgc ttt ggg cac att tgc agc agc cac ccc tcc tgc tgc tgc     1551
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Lys Lys Arg Phe Gly His Ile Cys Ser Ser His Pro Ser Cys Cys Cys
            480                 485                 490

acc gtc tcc aac agc tcc tgg aac tgc gac ggg gag gtc ctg cac agc     1599
Thr Val Ser Asn Ser Ser Trp Asn Cys Asp Gly Glu Val Leu His Ser
        495                 500                 505

cct gcc atc gag gtc aga gtc cac tgc cag ctg gtt cga ctc ttt gca     1647
Pro Ala Ile Glu Val Arg Val His Cys Gln Leu Val Arg Leu Phe Ala
    510                 515                 520

cga gga att gaa gag aat ccg aag cca gac tca cac agc tga             1689
Arg Gly Ile Glu Glu Asn Pro Lys Pro Asp Ser His Ser
525                 530                 535

gaagccggcg tcctgctctc gaactgggaa agtgtgaaaa ctatttaaga taattattac   1749

agaccaatta tgttgatata tacatttaaa tgtagaaatt tatttttgat agttaaatct   1809

tgattttaga agaaaaccct tttgtcaaca attttgtgta catatttggc attttcagtt   1869

ctgtacgcat ctgcgggttg cagcccacgc cgcttactct cagcggatgc agctgctcac   1929

ttgggggcac tggcctctta ggttttaacg atgtcaacag tgtagtttag aaaatggccc   1989

gttagtggct ctattgcaat aatgttaggg acattatatg atttccacgc aggtcacacc   2049

atctgggcct gaggtagcag tgggtcactt tgatccactt tgcaggactt attctgtaac   2109

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tcgtttacgg ttaggacatc gctgcttgat ccttacaata ctgtgcaact gcaatgcaac   2229

gtggccctgc ttcaggtgat ccgcgggagg ggcctccacg ccagcgccgg gaaggctgct   2289

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acaggcagca tccattttca gaatttcctg gcattgattt atattttgaa gcatacagga   3069

aacttctcgt ttcctcgttt agccccaccc agatcaggtg aaagggcagc tttaatggtg   3129
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gttttatgg accacattat cagagagcac tgtgcaagcc aaatggttca ataatgaatg 3189

aaaattctgg gtgtaaagag taaatatgcc ctggctcttt ctaccaatgt ttgctcctgg 3249

ttggaaagaa accaaagatt taagacgggc tgctcttcca gactggctgt gcctgcctgt 3309

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<212> PRT
<213> Homo sapiens

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20 25 30

Arg Trp Trp Arg Ser Pro Gly Pro Gly Ala Gly Ala Pro Gly Ala Asp
35 40 45

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Ala Cys Ser Val Pro Val Ser Glu Ile Ile Ala Val Glu Glu Thr Asp
    50                  55                  60

Val His Gly Lys His Gln Gly Ser Gly Lys Trp Gln Lys Met Glu Lys
65                  70                  75                  80

Pro Tyr Ala Phe Thr Val His Cys Val Lys Arg Ala Arg Arg His Arg
                85                  90                  95

Trp Lys Trp Ala Gln Val Thr Phe Trp Cys Pro Glu Glu Gln Leu Cys
            100                 105                 110

His Leu Trp Leu Gln Thr Leu Arg Glu Met Leu Glu Lys Leu Thr Ser
        115                 120                 125

Arg Pro Lys His Leu Leu Val Phe Ile Asn Pro Phe Gly Gly Lys Gly
    130                 135                 140

Gln Gly Lys Arg Ile Tyr Glu Arg Lys Val Ala Pro Leu Phe Thr Leu
145                 150                 155                 160

Ala Ser Ile Thr Thr Asp Ile Ile Val Thr Glu His Ala Asn Gln Ala
                165                 170                 175

Lys Glu Thr Leu Tyr Glu Ile Asn Ile Asp Lys Tyr Asp Gly Ile Val
            180                 185                 190

Cys Val Gly Gly Asp Gly Met Phe Ser Glu Val Leu His Gly Leu Ile
        195                 200                 205

Gly Arg Thr Gln Arg Ser Ala Gly Val Asp Gln Asn His Pro Arg Ala
    210                 215                 220

Val Leu Val Pro Ser Ser Leu Arg Ile Gly Ile Ile Pro Ala Gly Ser
225                 230                 235                 240

Thr Asp Cys Val Cys Tyr Ser Thr Val Gly Thr Ser Asp Ala Glu Thr
                245                 250                 255

Ser Ala Leu His Ile Val Val Gly Asp Ser Leu Ala Met Asp Val Ser
            260                 265                 270

Ser Val His His Asn Ser Thr Leu Leu Arg Tyr Ser Val Ser Leu Leu
        275                 280                 285

Gly Tyr Gly Phe Tyr Gly Asp Ile Ile Lys Asp Ser Glu Lys Lys Arg
    290                 295                 300
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Trp Leu Gly Leu Ala Arg Tyr Asp Phe Ser Gly Leu Lys Thr Phe Leu
305                 310                 315                 320

Ser His His Cys Tyr Glu Gly Thr Val Ser Phe Leu Pro Ala Gln His
                325                 330                 335

Thr Val Gly Ser Pro Arg Asp Arg Lys Pro Cys Arg Ala Gly Cys Phe
            340                 345                 350

Val Cys Arg Gln Ser Lys Gln Gln Leu Glu Glu Glu Gln Lys Lys Ala
        355                 360                 365

Leu Tyr Gly Leu Glu Ala Ala Glu Asp Val Glu Glu Trp Gln Val Val
    370                 375                 380

Cys Gly Lys Phe Leu Ala Ile Asn Ala Thr Asn Met Ser Cys Ala Cys
385                 390                 395                 400

Arg Arg Ser Pro Arg Gly Leu Ser Pro Ala Ala His Leu Gly Asp Gly
                405                 410                 415

Ser Ser Asp Leu Ile Leu Ile Arg Lys Cys Ser Arg Phe Asn Phe Leu
            420                 425                 430

Arg Phe Leu Ile Arg His Thr Asn Gln Gln Asp Gln Phe Asp Phe Thr
        435                 440                 445

Phe Val Glu Val Tyr Arg Val Lys Lys Phe Gln Phe Thr Ser Lys His
    450                 455                 460

Met Glu Asp Glu Asp Ser Asp Leu Lys Glu Gly Gly Lys Lys Arg Phe
465                 470                 475                 480

Gly His Ile Cys Ser Ser His Pro Ser Cys Cys Cys Thr Val Ser Asn
                485                 490                 495

Ser Ser Trp Asn Cys Asp Gly Glu Val Leu His Ser Pro Ala Ile Glu
            500                 505                 510

Val Arg Val His Cys Gln Leu Val Arg Leu Phe Ala Arg Gly Ile Glu
        515                 520                 525

Glu Asn Pro Lys Pro Asp Ser His Ser
    530                 535
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<211> 562
<212> PRT
<213> Homo sapiens

<400> 11

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His Glu Ala Ala Asn Gly Pro Ala Pro Leu Gly Val Arg Ala Pro Pro
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Ala Trp Arg Thr Ser Pro Ala Ala Glu Met Gly Ala Thr Gly Ala Ala
            20                  25                  30

Glu Pro Leu Gln Ser Val Leu Trp Val Lys Gln Gln Arg Cys Ala Val
        35                  40                  45

Ser Leu Glu Pro Ala Arg Ala Leu Leu Arg Trp Trp Arg Ser Pro Gly
    50                  55                  60

Pro Gly Ala Gly Ala Pro Gly Ala Asp Ala Cys Ser Val Pro Val Ser
65                  70                  75                  80

Glu Ile Ile Ala Val Glu Glu Thr Asp Val His Gly Lys His Gln Gly
                85                  90                  95

Ser Gly Lys Trp Gln Lys Met Glu Lys Pro Tyr Ala Phe Thr Val His
            100                 105                 110

Cys Val Lys Arg Ala Arg Arg His Arg Trp Lys Trp Ala Gln Val Thr
        115                 120                 125

Phe Trp Cys Pro Glu Glu Gln Leu Cys His Leu Trp Leu Gln Thr Leu
    130                 135                 140

Arg Glu Met Leu Glu Lys Leu Thr Ser Arg Pro Lys His Leu Leu Val
145                 150                 155                 160

Phe Ile Asn Pro Phe Gly Gly Lys Gly Gln Gly Lys Arg Ile Tyr Glu
                165                 170                 175

Arg Lys Val Ala Pro Leu Phe Thr Leu Ala Ser Ile Thr Thr Asp Ile
            180                 185                 190

Ile Val Thr Glu His Ala Asn Gln Ala Lys Glu Thr Leu Tyr Glu Ile
        195                 200                 205

Asn Ile Asp Lys Tyr Asp Gly Ile Val Cys Val Gly Gly Asp Gly Met
    210                 215                 220

Phe Ser Glu Val Leu His Gly Leu Ile Gly Arg Thr Gln Arg Ser Ala
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225 230 235 240

Gly Val Asp Gln Asn His Pro Arg Ala Val Leu Val Pro Ser Ser Leu
245 250 255

Arg Ile Gly Ile Ile Pro Ala Gly Ser Thr Asp Cys Val Cys Tyr Ser
260 265 270

Thr Val Gly Thr Ser Asp Ala Glu Thr Ser Ala Leu His Ile Val Val
275 280 285

Gly Asp Ser Leu Ala Met Asp Val Ser Ser Val His His Asn Ser Thr
290 295 300

Leu Leu Arg Tyr Ser Val Ser Leu Leu Gly Tyr Gly Phe Tyr Gly Asp
305 310 315 320

Ile Ile Lys Asp Ser Glu Lys Lys Arg Trp Leu Gly Leu Ala Arg Tyr
325 330 335

Asp Phe Ser Gly Leu Lys Thr Phe Leu Ser His His Cys Tyr Glu Gly
340 345 350

Thr Val Ser Phe Leu Pro Ala Gln His Thr Val Gly Ser Pro Arg Asp
355 360 365

Arg Lys Pro Cys Arg Ala Gly Cys Phe Val Cys Arg Gln Ser Lys Gln
370 375 380

Gln Leu Glu Glu Glu Gln Lys Lys Ala Leu Tyr Gly Leu Glu Ala Ala
385 390 395 400

Glu Asp Val Glu Glu Trp Gln Val Val Cys Gly Lys Phe Leu Ala Ile
405 410 415

Asn Ala Thr Asn Met Ser Cys Ala Cys Arg Arg Ser Pro Arg Gly Leu
420 425 430

Ser Pro Ala Ala His Leu Gly Asp Gly Ser Ser Asp Leu Ile Leu Ile
435 440 445

Arg Lys Cys Ser Arg Phe Asn Phe Leu Arg Phe Leu Ile Arg His Thr
450 455 460

Asn Gln Gln Asp Gln Phe Asp Phe Thr Phe Val Glu Val Tyr Arg Val
465 470 475 480

```
Lys Lys Phe Gln Phe Thr Ser Lys His Met Glu Asp Glu Asp Ser Asp
                485                 490                 495

Leu Lys Glu Gly Gly Lys Lys Arg Phe Gly His Ile Cys Ser Ser His
            500                 505                 510

Pro Ser Cys Cys Cys Thr Val Ser Asn Ser Ser Trp Asn Cys Asp Gly
        515                 520                 525

Glu Val Leu His Ser Pro Ala Ile Glu Val Arg Val His Cys Gln Leu
    530                 535                 540

Val Arg Leu Phe Ala Arg Gly Ile Glu Glu Asn Pro Lys Pro Asp Ser
545                 550                 555                 560

His Ser
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<210> 12
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<212> DNA
<213> Homo sapiens

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